

METHOD FOR PRODUCTION OF PHYTOALEXINS

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Abstract of WO03077881

The invention relates to a topical composition, in particular a cosmetic rich in metabolites produced by dedifferentiated plant cells elicited in vitro, then dried, milled and dispersed in said composition. The invention further relates to a method for preparation of phytoalexin(s).

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Claims < RTI ID=0.0> 1. < /RTI> Composition for topical application, in particular cosmetic composition, containing dédifférenciées vegetable cells, characterized in that the composition contains at least a broyat vegetable cells dédifférenciées and élicitées in vitro culture to synthesize at least a phytoalexine, this elicitation to synthesize at least a phytoalexine being advantageously operated after a stage of in vitro culture of vegetable cells without elicitation, in which the aforementioned broyat containing at least a phytoalexine includes/understands at least 95%, advantageously at least 97%, preferably at least 99% in weight of the whole of the matters dry resulting from the vegetable cells crushed dédifférenciées and élicitées in vitro, the aforementioned broyat being dispersed in the aforementioned composition or being in a form ready to be dispersed in the aforementioned composition.

2. Composition according to claim 1, characterized in that the broyat of vegetable cells dédifférenciées and élicitées in vitro includes/understands particles coming from the vacuoles, of the particles coming from the cytoplasm and the particles coming from the cellulose membrane pecto, the aforementioned broyat containing at least 0,1% in weight of phytoalexine (S).

3. Composition according to the claim 1 or 2, characterized in that the broyat is a broyat cells dédifférenciées and élicitées in vitro, the aforementioned cells being at least partially dried.

4. Composition according to claim 3, characterized in that the broyat is a broyat cells dédifférenciées and élicitées in vitro, the aforementioned cells being appreciably completely dried, preferably freeze-dried, and then crushed.

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5. Composition according to any of claims 1 to 4, characterized in that it contains from 0,005 to 25% in weight, advantageously from 0,005 to 5% in weight of broyat of vegetable cells dédifférenciées and élicitées in vitro, this weight being calculated in dry form.

< RTI ID=0.0> 6. < /RTI> Composition according to one of claims 1 to 5, characterized in that it contains a dry broyat or appreciably dryness of vegetable cells dédifférenciées and élicitées in vitro, the aforementioned broyat dry or appreciably dry containing a water content lower than 25% in weight, advantageously lower than 15% in weight, preferably lower than 10% in weight.

7. Composition according to one of claims 1 to 6, characterized in that the broyat has an average granulometry of solid particles lower than 100 < RTI ID=0.0> U. m, < /RTI> advantageously lower than < RTI ID=0.0> 10, um, < /RTI> preferably lower than 1 < RTI ID=0.0> llm. < /RTI>

8. Composition according to claim 7, characterized in that the particles of the broyat have a granulometry such as 90% in weight of the particles have a granulometry included/understood in the beach granulometry average-25% until average granulometry + 25%.

9. Composition according to any of the preceding claims, characterized in that the aforementioned broyat of vegetable cells dédifférenciées and élicitées in vitro contains at least a phytoalexine synthesized by in vitro elicitation of the dédifférenciées vegetable cells.

10. Composition according to any of the preceding claims, characterized in that the aforementioned broyat of dédifférenciées and élicitées vegetable cells is a broyat vegetable cells dédifférenciées and élicitées in vitro by an agent in the culture medium. the aforementioned broyat being appreciably free of the aforesaid agent after elicitation.

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11. Composition according to the preceding claim, characterized in that the dédifférenciées cells are élicitées in vitro by an agent bird.

< RTI ID=0.0> 12. < /RTI> Composition according to any of the preceding claims, characterized in that the aforementioned broyat of vegetable cells dédifférenciées and élicitées in vitro contains at least a terpenic or tannic or polyphenolic compound, the aforementioned compound being synthesized by in vitro elicitation of the vegetable cells dédifférenciées in their culture medium.

13. Composition according to any of the preceding claims, characterized in that the broyat of vegetable cells dédifférenciées and élicitées in vitro appears as a viscous suspension or a gel or an appreciably dry powder, the aforementioned suspension, freezing or powder being advantageously in a form ready to be dispersed in the composition.

14. Composition according to any of the preceding claims, characterized in that the broyat of cells is a broyat cells of vine dédifférenciées and élicitées in vitro.

15. Composition according to any of the preceding claims, characterized in that it contains a broyat cells différenciées, cultivated and élicitées in their in vitro culture medium, the aforementioned broyat being advantageously appreciably free from culture medium.

16. Composition according to any of the preceding claims, characterized in that it contains a broyat cells différenciées and élicitées in vitro, the aforementioned broyat containing at least 0,1% in weight of stilbenes compared to the dry weight of the différenciées cells élicitées crushed, in particular at least 0,2% in weight of stilbenes compared to the dry weight of the différenciées cells élicitées crushed, preferably at least 0,5% in weight of stilbenes compared to the dry weight of the différenciées cells élicitées crushed.

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17. Composition according to any of the preceding claims, characterized in that the broyat of élicitées différenciées cells results from the culture of différenciées vegetable cells, élicitées then dried of at least a species chosen among Salvia, Coleus, Rosmarinus, Ginkgo, Cannabis, Colchicum, Gloriosa, Asparagus, Argan, Glycine, Medicago, Mungo, Erythrina, Oenothera, Papaver, Atropa, Datura, Solanum, Borago, Reseda, Amsonia, Catharantus, Pilocarpus, Digitalis, Coffea, Theobroma, Jasminum, Capsicum, Iris, vine, taxus, sequoia, chlorophytum, cocoa, psoralea corylifolia, vitex negundo, will commiphora wighii, eucalyptus punctata, lavandula angustifolia, citrus silt, vanilla planifolia, marrubium vulgare, pilocarpus jaborandi, pinks, betula, tea and the mixtures of cells of such species.

18. Carried out of preparation of a composition topic use according to any of the preceding claims, characterized in that one puts of the vegetable cells différenciées in a culture medium so as to allow a growth of the cells, in what one elicit the aforementioned vegetable cells differentiated in their culture medium for one period of time sufficient for the synthesis from a sufficient quantity from metabolites, and in what one mixes the élicitées vegetable cells of the culture medium with one or more excipients to prepare a cosmetic composition, the cells being crushed before and/or after their mixture with one or more excipients and/or before and/or after a stage of drying.

19. Proceeded according to claim 18, characterized in that one subjects the aforementioned élicitées cells in vitro to a drying, follow-up of a crushing.

20. Proceeded according to any of claims 18 and 19, characterized in that one elicit cells in their in vitro culture medium by means of an agent, which after extraction of the élicitées cells of the culture medium by preserving the membrane structure of the cells does not find in the élicitées cells.

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21. Proceeded in which différenciées vegetable cells are put in in vitro culture, élicitées in in vitro culture medium, dried, then crushed (possibly after one or more stages of washing/drying) and dispersed in a composition of treatment of the human body.

22. Proceeded according to the preceding claim, characterized in that the cells are dried by freeze-drying before being subjected to a crushing.

23. Proceeded according to the claim 21 or 22, characterized in that one uses an agent or a means of elicitation not forming an impurity in the dried broyat of différenciées and élicitées cells.

24. Process of obtaining phytoalexine (S), in which: < RTI ID=0.0> - on< /RTI> puts in a culture medium of the vegetable cells différenciées, - after and/or during the culture, one elicit the cells différenciées in the culture medium, < RTI ID=0.0> - on< /RTI> separate the cells différenciées and élicitées from the culture medium, < RTI ID=0.0> - on< /RTI> subjects possibly the différenciées cells élicitées to one or more washings, < RTI ID=0.0> - on< /RTI> dry advantageously, preferably one freeze-dries, the différenciées and élicitées cells, < RTI ID=0.0> - on< /RTI> crush the différenciées cells, élicitées so as to form a broyat, and < RTI ID=0.0> - on< /RTI> the broyat subjects to an extraction to extract one or more phytoalexines from the broyat, possibly after a stage of handing-over of the cells crushed in a medium, in particular an aqueous and/or alcoholic medium.

25. Process following any of claims 18 to 24, characterized in that one controls the stage of elicitation of the aforesaid vegetable cells differentiated in their in vitro culture medium, so as to obtain a medium containing at least 0,1% in weight of stilbenes compared to the dry weight of the différenciées and élicitées vegetable cells, in particular at least 0,2% in weight of stilbenes

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compared to the dry weight of the cells, preferably at least 0,5% in weight of stilbenes compared to the dry weight of the cells.

26. Proceeded according to any of claims 18 to 25, characterized in that the broyat results from the culture of différenciées vegetable cells, élicitées then dried of the species Salvia, Coleus, Rosmarinus, Ginkgo, Cannabis, Colchicum, Gloriosa, Asparagus, Arganier, Glycine, Medicago, Mungo, Erythrina, Oenothera, Papaver, Atropa, Datura, Solanum, Borago, Reseda, Amsonia, Catharantus, Pilocarpus, Digitalis, Coffea, Theobroma, Jasminum, Capsicum, Iris, vine, taxus, sequoia, chlorophytum, cocoa, psoralea corylifolia, vitex negundo, will commiphora wighii, eucalyptus punctata, lavandula angustifolia, citrus silt, vanilla planifolia, marrubium vulgare, pilocarpus jaborandi, pinks, betula, tea, and their mixtures.

27. Broyat of différenciées vegetable cells, élicitées in in vitro culture medium, then dried, in which the aforementioned broyat containing at least a phytoalexine includes/understands at least 95% in weight, advantageously at least 97% in weight, preferably at least < RTI ID=0.0> 99%< /RTI> in weight of the whole of the matters dry resulting from the vegetable cells crushed différenciées and élicitées in vitro, the aforementioned broyat being in a form ready to be dispersed in a cosmetic and/or pharmaceutical composition.

28. Broyat following claim 27, characterized in that it is free from agent of elicitation and/or culture medium.

29. Broyat of vegetable cells according to the claim 27 or 28, characterized in that it presents an average granulometry lower than $< RTI\ ID=0.0> 101\mu m. < /RTI>$

30. Broyat of vegetable cells according to one of claims 27 to 29, characterized in that it contains at least 0,1% in weight of stilbenes compared to the dry weight of the cells, in particular at least 0,2% in weight of stilbenes by

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report/ratio with the dry weight of the cells, preferably at least 0,5% in weight of stilbenes compared to the dry weight of the cells.

31. Composition for topical application, in particular cosmetic composition, containing *dédifférenciées* vegetable cells, characterized in that the composition contains at least a broyat containing at least a phytoalexine, the aforementioned broyat including/understanding at least 95%, advantageously at least 97%, preferably at least 99% in weight of the whole of the matters dry resulting from the crushed *dédifférenciées* and *élicitées* vegetable cells in vitro, in which the broyat contains at least 0,1% in weight of stilbenes compared to the dry weight of the cells, in particular at least 0,2% in weight of stilbenes compared to the dry weight of the cells, preferably at least 0,5% in weight of stilbenes compared to the dry weight of the cells.

32. Composition according to the claim $< RTI\ ID=0.0> 31, < /RTI>$ characterized in that the broyat is a broyat of one or *dédifférenciées* vegetable cells, *élicitées* then dried selected among the group made up of the following species: Salvia, Coleus, Rosmarinus, Ginkgo, Cannabis, Colchicum, Gloriosa, Asparagus, Argan, Glycine, Medicago, Mungo, Erythrina, Oenothera, Papaver, Atropa, Datura, Solanum, Borago, Reseda, Amsonia, Catharantus, Pilocarpus, Digitalis, Coffea, Theobroma, Jasminum, Capsicum, Iris, vine, taxus, sequoia, chlorophytum, cocoa, psoralea corylifolia, vitex negundo, will commiphora wighii, eucalyptus punctata, lavandula angustifolia, citrus silt, vanilla planifolia, marrubium vulgare, pilocarpus jaborandi, pinks, betula, tea, and their mixtures.



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PROCESS OF OBTAINING PHYTOALEXINES

The invention has as an aim a composition with use topic, in particular cosmetic, rich in metabolites produced by dédifférenciées vegetable cells. In particular, the invention has as an aim a composition containing of the vegetable cells dédifférenciées and élicitées then dried partially or completely preferably, freeze-dried, crushed and dispersed in this composition.

By dédifférenciées vegetable cells, one understands any vegetable cell not presenting none characters of a specialization particular and able to live by itself and not in dependence with other cells.

The dédifférenciées vegetable cells can be obtained starting from vegetable material resulting from whole plant or part of plant like the sheets, the stems, the flowers, the petals, the roots, the fruits, their skin, the envelope protecting them, seeds, the anthères, the sap, the spines, the buds, the bark, bays, and of the mixtures of those.

Preferentially, the dédifférenciées vegetable cells are obtained starting from bark, of sheets, buds and the skin of the fruits, in particular of the cuticule of the fruit.

The vegetable cells dédifférenciées usable according to the invention can be obtained starting from plants obtained by culture in vivo or resulting from in vitro culture.

By culture in vivo one understands any culture of the traditional type < RTI ID=0.0> it est< /RTI> with saying in ground to the free air or greenhouse or except ground or in hydroponic medium.

By in vitro culture, one understands the whole of the known techniques of the specialist of the profession which allows in an artificial way obtaining of a plant or part of a plant. Pressure of selection imposed by the physico conditions

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chemical at the time of the growth of the vegetable cells in vitro allows to obtain a standardized vegetable material, free from contaminations and available throughout the year contrary to the crop plants in vivo.

Preferentially according to the invention one uses dédifférenciées vegetable cells resulting from in vitro culture.

The vegetable cells dédifférenciées usable according to the invention can be obtained by any known method of former art. In this respect one can quote the methods described by E. F. George and P. D. < RTI ID=0.0> Sherrington< /RTI> in Seedling Propagation by tissue culture, handbook and directory off commercial laboratories < RTI ID=0.0> (Exegetics< /RTI> Ltd 1984).

The culture media usable according to the invention are those generally known of the specialist of the profession. One can quote as examples the mediums of Gamborg, < RTI ID=0.0> Murashige< /RTI> and Skoog, Heller, White etc?. One will find in " Seedling Culture Media: and formulations use " of E. F. George, DJM Puttock and H. J George (Exegetics Ltd 1987, volume 1 & 2) of complete descriptions of these mediums.

Preferentially according to the invention one prepares the dédifférenciées vegetable cells cultivated on medium of Murashige and Skoog.

State of the art One knows by the document FR 2795637 a cosmetic composition containing an extract of vegetable cells dédifférenciées to avoid the problems of odor. This composition contains an extract of dédifférenciées vegetable cells but not élicitées, so that this composition is low in secondary metabolites or phytoalexines, even is appreciably free from such compounds. Moreover this document describes the use of aqueous extract obtained after crushing of the cells in their culture medium then elimination of the suspended particles with an inevitable loss of the metabolites related to the suspended particles. In order to eliminate the proteases and in particular the oxydases this document also recommends the use of filters capturing them

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molecules of a molecular weight higher than 100.000 let us daltons thus losing in the final extract all the metabolites superiors with this weight and which can prove of a great interest for cosmetic industry. Moreover in order to eliminating the problems due to oxidation the document recommends the addition of stabilizing, in particular the cysteine and/or of the sulphur derivatives what involves necessarily a less purity of the extract with subsequent stages of filtration. The methods described in this document require the implementation of means complicated for obtaining extracts of which, so much the purity (many additives), which quality and concentration (in metabolites) < RTI ID=0.0> est< /RTI> pas optimale. Moreover many stages necessary to obtaining of the extracts of this process induce high costs and the risk of contaminations of share many handling and additives.

One knows the cultures of différenciées cells, in addition one knows the mechanisms of elicitation of these cells followed by stages of extractions and various filtrations followed by freeze-drying in order to incorporate the extracts obtained in a cosmetic or pharmaceutical preparation. Such processes for example are described in US 4,241, 536; EP 378 < RTI ID=0.0> 921, < RTI ID=0.0> WO 88/00968, EP < RTI ID=0.0> 1 203.811, < RTI ID=0.0> etc for species of various plants. The contents of these documents are built-in in present description by reference to describe culture media, species of plants, éliciteurs possible, etc

Nowadays, in spite of competences and the knowledge to make industries in the field of the vegetable extraction, and in spite of progress of the organic chemistry, several stages of extraction are essential to obtain a raw material vegetable.

Several disadvantages are charged to these extractions: - loss of the tertiary structure of the insulated molecules, - presence of various solvents on the level of the finished product, - heterogeneity of the substrates requiring of the fine extractions calling upon increasingly toxic solvents,

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- quality of the extract according to the physiological state of the plant at the time of harvest, - production of the extract limited according to the seasons,

Vis-a-vis these limiting factors and with the renewed interest of the consumers for all that is of natural origin, several attempts at obtaining cells were carried out. Thus, to date, two major processes were exploited: < RTI ID=0.0> - La < RTI ID=0.0> culture of cells starting from unicellular organizations or < RTI ID=0.0> micro-organisms, < RTI ID=0.0> not very original technique basing itself on the reproduction of the living conditions normal. However these organizations are primitive and do not develop secondary metabolism, source of the active ingredients most interesting.

- Obtaining the cells starting from fruits (fresh cells) after enzymatic digestion. Limits of this process resident in the fact that the fruits are not aseptic and that they can contain residues of pesticides (fungicidal, weedkillers, insecticides,?). D'autre part, les enzymes (cellulases, pectinases,...) utilisées en quantité importante (2%, P/P) pour la digestion des parois végétales et l'obtention des cellules sans paroi (protoplastes) se retrouvent dans le produit fini. In addition, the enzymes used can deteriorate the quality of the metabolites.

Lastly, the use of this technique only makes it possible to recover protoplasts (cells without cellular wall), fragile structures not being able to direct their metabolism.

The inventors developed a technology innovating and controlled, guaranteeing the quality and the authenticity of the products. It is about the setting in culture of cells of différenciées higher plants.

Indeed, and for the first time, an industrial process makes it possible to obtain cells starting from higher plants by a process without any modification of their genetic inheritance, making it possible the cell to keep its physiological characteristics.

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The maintenance of the various stocks is ensured by regular road repairs, with a total control of the various conditions of culture.

Interest of this process and that it allows the culture of vegetable cells différenciées in great volumes while meeting the needs for industry, in particular: < RTI ID=0.0> - Le < RTI ID=0.0> respect of the tertiary structure of the molecules, - the absence of solvent and residues, - homogeneity of the substrates, < RTI ID=0.0> - La < RTI ID=0.0> production continues independently of the cycle of the seasons, < RTI ID=0.0> - La < RTI ID=0.0> conservation of the biological and physiological characteristics without addition of conservative, - the total absence of pollutants, < RTI ID=0.0> - La < RTI ID=0.0> production standardized and reproducible as for the quality and the concentration of the metabolites, - the use of these vegetable suspensions after direct freeze-drying with use of lower temperature < RTI ID=0.0> in-30 C. < RTI ID=0.0> This technique allows obtaining a very fine powder being able to be dispersed in cosmetic compositions (creams, pomades, lotions?). These cells are likely to directly release the active ingredients which they contain, without passage by an extraction using organic solvents (elimination of the risks of residues).

However, it is preferable to subject the product of freeze-drying to a crushing to avoid any agglomeration of particles.

- The use of the cellular extracts only after sonification and centrifugation.

This technology brings an alternative useful and innovating for the conventional extractions by solvents. The possibility of directing in a natural way (elicitation) the synthesis of the metabolites without attacking the genetic integrity of the cells, represents a guarantee of quality and authenticity.

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In a way completely surprising the inventors discovered that < RTI ID=0.0> one < RTI ID=0.0> could directly incorporate or disperse the cells after elicitation, drying and crushing in a cosmetic and/or pharmaceutical composition. The composition according to the invention then contains membranes of cells, organoids cytoplasmic and vacuolar matter. This process has in particular the advantage of decontaminating the oxidizing enzymes without additions of additives or chemicals. Un autre aspect de l'invention permet de concentrer et de diriger la production de phytoalexines sans pertes quantitatives ou qualitatives dues aux extractions et aux filtrages. A particular aspect of the invention is that it avoids the stages of extraction and filtering and allows obtaining a broyat of cells stripped of additives, solvents and residues, the aforementioned broyat being able directly to be dispersed in a cosmetic composition.

The composition according to the invention contains at least a broyat vegetable cells différenciées and élicitées in vitro culture to synthesize at least a phytoalexine, this elicitation to synthesize at least a phytoalexine being advantageously operated after a stage of in vitro culture of vegetable cells without elicitation, in which the aforementioned broyat containing at least a phytoalexine includes/understands at least 95%, advantageously at least 97%, preferably at least 99% in weight of the whole of the matters dry resulting from the vegetable cells crushed différenciées and élicitées in vitro, the aforementioned broyat being dispersed in the aforementioned composition or

being in a form ready to be dispersed in the aforementioned composition. In particular, the broyat appreciably contains all the dry matters (after extraction of water present in the cells) of the différenciées and élicitées vegetable cells crushed. Such a broyat rich in phytoalexine compared to the contents of the not élicitées cells contains however all the natural matters present in the cells.

The goal of this invention is amongst other things to propose a simple process excluding employment from additives and chemicals and respecting the natural character of the cells obtained. Moreover, the elicitation by average physiques makes it possible to direct and concentrate the production of metabolites sought by

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cosmetic industry. The particular aspect of the invention by respecting the integrity of the cells obtained and by élicitant them makes it possible on the one hand to optimize the concentration and the quality of the metabolites obtained, on the other hand to solve the problems of oxidation by inactivant the enzymes by a simple drying (freeze-drying) without addition of additives and losses due to filtrations and finally allows the dispersion of the broyat obtained directly in a cosmetic preparation.

Par élicitation dans le milieu de culture, on entend la mise en oeuvre d'un stress ou d'une agression (biologique, chimique ou physique) sur les cellules dans leur milieu de culture afin de provoquer un ou plusieurs mécanismes de défense.

Throughout their development, the plants are subjected to continual aggressions on behalf of their environment. However, they are shown often able to resist naturally these aggressions external thanks to the presence or activation of mechanisms of defence < RTI ID=0.0 > (Hammond-Kosack < /RTI > and Jones, 1996). Thus, although some of these mechanisms are constitutive and provide a physical and chemical barrier to an aggression, others are induced only after one attack by a vermin.

As soon as a plant detects pathogenic, it sets up one of the most effective systems of natural defense: the response of over-sensitiveness. On the level of the site of penetration of pathogenic, this reaction, rapid and force, result in the death of the first infected cells and the appearance of a small zone necrotic, thus isolating the cells attacked from the remainder of the plant (Dangl and Al, 1996; Lamb and Dixon, 1997). The release of this answer depends on a specific recognition of the pathogenic agent by the plant host. Indeed, the attacked plant recognizes via a protein (receiver) a protein produced by pathogenic, named the elicitor. At the plant, the genes coding for receiving proteins are called genes of resistance (genes R), and for the pathogenic one, the genes coding for the molecules élicitrices are named genes of avirulence (Avr gene): one speaks then here about a relation gene

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for gene or relation R-Avr (Hammond-Kosack, 1996). Other molecules, qualified general éliciteurs, are also able to initiate (in a way less specific than the éliciteurs quoted previously) this defence reaction of the plant host. Those, are generally < RTI ID=0.0 > oligosaccharides < /RTI > released by the pathogenic one (exogenic éliciteurs) or the vegetable cell (endogenous éliciteurs), (Scheel and Parker, 1996).

With this reaction of over-sensitiveness the activation of an intense defence reaction in the cells close to the infected zone succeeds, it is the local reaction. Lastly, the emission of various signals of alarm towards all the other bodies of the plant, which enables him to react more quickly and more effectively at the time of a new aggression, one speaks then about systemic reaction.

During these reactions, three categories of systems of defense can be activated: < RTI ID=0.0 > - la < /RTI > formation of a skin of cicatrization and reinforcement of the walls (lignification?) (Dai and Al, 1995); < RTI ID=0.0 > - la < /RTI > protein synthesis of defense or Pathogenesis Related (PRs) proteins discovered in 1970 at the Tobacco. Among these PRs one finds, for example, of the inhibitors of protease (Ryan, 1992), of the hydrolytic enzymes, like the chitinases or the < RTI ID=0.0 > B-1, < /RTI > 3 glucanases < RTI ID=0.0 > (Derckel < /RTI > and Al, 1996; Robinson and Al, 1997, Kraeva and Al, 1998; Salzman et al., 1998 ; Renault and Al, 2000); < RTI ID=0.0 > - et < /RTI > the synthesis of secondary metabolites of phytoalexines type. Among, the secondary metabolites, more than 300 phytoalexines were already characterized. They belong to a broad spectrum of different chemical classes among which one will be able to quote coumarins, the benzofurans, terpenes, alkaloids, certain polyphenols (Smith, 1996)?

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The installation of the defence reactions of the plants implies a whole panoply of signals of transduction leading to the fast induction of the gene expression of defense. Thus, the recognition of pathogenic by the plant host will activate a whole cascade of signals in the cells attacked such as protein phosphorylation by proteins kinases, ionic flows of species < RTI ID=0.0 > (Ca²⁺), < /RTI > formation of reactive oxygenated species (Dimensioned and Hahn, < RTI ID=0.0 > 1994; Shibuya < /RTI > and Al, 1996; Benhamou, 1996)?

Moreover, the attacked cells are able to produce alarms transmitted to the close cells (local reaction) like with all the plant thus generating, like statement in the preceding paragraph, the phenomenon of systemic reaction.

The systemic mechanism of resistance more studied is the phenomenon of SAR or systemic acquired resistance. Le terme de SAR a été défini par Ross en 1961. It describes the appearance of resistance of a consecutive plant to an attack by pathogenic, as well in the parts infected as in the healthy parts of the plant. It develops, in general, after the appearance of lesions necrotic around the site of inoculation. This response of localised over-sensitiveness restricted the pathogenic one in, and around the site of infection, and seems to make the plant more resistant to the aggressions by various organizations (Ryals and Al, 1996). How the distant parts of the site of infection are they able to acquire this resistance? It is into 1966 that Ross developed the idea of the existence of molecules signal which, with weak concentrations, would be able to activate mechanisms of defence in distant fabrics of the infected zone.

Three types of molecules can intervene at the plants as alarm on the levels will intra and intercellular, with short or long distance: salicylic acid, ethylene and < RTI ID=0.0 > jasmonates. < /RTI >

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The mechanism of transduction more studied and the best known one in the development of the SAR are that implying the salicylic acid (ACE) (Delaney and Al, 1994; Ryals and Al, 1996).

On the one hand, the acquisition of resistance of certain plants is directly related to an increase in the endogenous synthesis of ACE or its derivatives (Malamy and Al, 1990; Métraux and Al, 1990; Rasmussen and Al, 1991; Smith-Becker and Al, 1998). In this type of answer, when the synthesis of ACE is initiated, the transduction of the signal would be carried out at long distance, that is to say by direct transport of < RTI ID=0.0> AS< /RTI> (Shulaev et al., 1995), soit par conversion de ce dernier en méthyl salicylate (MeSa), molécule-signal volatile pouvant entraîner l'apparition de résistance dans les tissus sains de la plante infectée mais aussi dans les plantes voisines (Shulaev et al., 1997). Moreover, the description of the part played by the ACE in the release of the SAR is related to the use of transgenic seedlings of Tobacco expressing the bacterial gene NahG. This gene, coding for salicylate hydroxylase, inactive < RTI ID=0.0> AS< /RTI> by converting it into catéchol, thus making the plants transformed unable to develop a SAR (Gaffney and Al, 1993).

In addition, certain studies show that the exogenic application of ACE is able to induce a resistance to various lesions caused by a bacterium, a virus or pathogenic. Thus, at the Tobacco, one observes a reduction of the symptoms related to the inoculation of the virus of the Mosaic of the Tobacco after treatment of the seedlings by ACE (White and Al, 1983). In the same way, < RTI ID=0.0> AS< /RTI> to stimulate the biosynthesis of various PRs proteins normally produced in the case of a SAR (Renault and Al, 1996 is able; Narusaka and Al, 1999).

Various studies showed that certain defence reactions could be activated independently of the presence of ACE. Thus, other types of molecules were recognized like molecule-signal of the SAR (Enyedi and Al, 1992; Pieterse and Al, 1999). These reactions would utilize mainly two vegetable growth regulators, the ethylene and the acid jasmonic (AJ),

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like signals of systemic induction of defense. Indeed, these two types of molecules can quickly be produced, in an endogenous way, when a plant is subjected to an aggression, and to thus involve the appearance of a resistance. Of, more they are also able to induce the expression of certain PRs < RTI ID=0.0> et/ou.< /RTI> production of phytoalexines.

The ethylene is a volatile phytohormone intervening in many physiological processes of the plants. Its role in the phenomena of vegetable defense was shown by several teams. Des études ont montré que l'attaque d'une plante par un pathogène ou un herbivore pouvait être corrélée à une induction ou une augmentation de la synthèse d'éthylène endogène dans la plante hôte < RTI ID=0.0> (O'Donnell</RTI> and Al, 1996; Pop and Al, 1997; Lund and Al, 1998). Moreover, just like < RTI ID=0.0> the ACE, < /RTI> the exogenic application of ethylene is also able to stimulate the synthesis of proteins PRs (Ward and Al, 1991; < RTI ID=0.0> Penninckx< /RTI> and Al, 1996; Yang and Al, 1997). Lastly, the protein activation of defense at Arabidopsis in response to pathogenic can be blocked in the case of mutant defective in the perception of this signal (Penninckx and Al, 1998).

The acid jasmonic and its methyl ester, the methyl jasmonate (MeJa), are of the natural compounds of type cyclopentanone analogues to animal prostaglandins by their biosynthesis and their function (Creelman and Al, 1992; Mason and Al, 1993; Sadka and Al, 1994). They derive from the fatty acids and are synthesized starting from oxidation lipoxygénase-dependent on the A-linoleic acid. One speaks about octadécanoïque way.

In addition to their role in various physiological functions (Stawick, 1992), these compounds were recently implied as molecules of indication intracellular synthesized in response to a stress, biotic or abiotic, and driving with the answers of defense of the plants, like the biosynthesis of phytoalexines or proteins of defense (Gundlach and Al, 1992; Bleichert et al., 1995; Creelman and Al, 1995; Doares and Al, 1995; Conconi and Al, 1996a and < RTI ID=0.0> 1996b; < /RTI> Ignatov and Al, 1996; Baldwin and Al, 1997).

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On the one hand, the application of MeJa, derived volatile from the acid jasmonic, induced production of many secondary, in vivo or in vitro metabolites (in cellular cultures), of which some play the part of made up of defense. Indeed, this ester is capable of éliciter the biosynthesis of furanocoumarines in the sheets of Celery (Miksch and Boland, 1996), of momilactone A in cellular cultures of Rice (Nojiri and Al, 1996), alkaloids of young seedlings or cellular cultures of Catharanthus roseus (Aerts and Al, 1996; Gantet and Al, 1997) and of young seedlings of Cinchona ledgeriana (Aerts and < RTI ID=0.0> Al, < /RTI> 1994 ; 1996).

Others derived precursors of the AJ, like the 12-oxo-phytodiénoïque acid, are able to act on the biosynthesis of many secondary metabolites.

The inductive effect of these compounds is often preceded by an activation by the form of genes or synthesis of enzymes intervening in biosynthesis by these various metabolites. Among these enzymes, one finds phenylalanine ammonialyase (STAKE), the 4-coumarate-CoA ligase (4CL), chalcone synthase (CHS), the < RTI ID=0.0> dihydroflavonol-4-reductase< /RTI> (DFR), the polyphenol oxydase (Po), the < RTI ID=0.0> mercaptol< /RTI> methyl transférse (BMT), tyrosin/doped décarboxylase (TYDC),? (Dittrich and Al, 1992; Gundlach et al., 1992 ; Mizukami and Al, 1993; Tamari and Al, 1995; < RTI ID=0.0> Ellard-Ivey< /RTI> and Al, 1996; < RTI ID=0.0> Facchini< /RTI> and Al, 1996; Ignatov and Al, 1996; Lee and Al, 1997; Yasaki and Al, < RTI ID=0.0> 1997; < /RTI> Constanbel and Ryan, 1998).

In addition, the exposure of plants to vapors of MeJa, allows éliciter mechanisms of defence similar to those induced by insects, herbivores, a wound or UV (Farmer and Ryan, 1990; 1992 ; Conconi and Al, 1996b; Ozawa and Al, 2000). Thus, the jasmonates, and in particular MeJa, are able to induce the biosynthesis of proteins of defense and stress, still called JIPs (Jasmonate Induced Proteins), (Reinbothe and Al, 1994).

Moreover, treatment of seedlings of Tomato, Potato or Alfalfa by MeJa, induced the expression of inhibitors of protease (Farmer and Ryan, < RTI ID=0.0> 1990, < /RTI>

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1992 ; Hildmann and Al, 1992; The Pena-Cortes and Al, < RTI ID=0.0> 1992; Lee< /RTI> et al., 1996), la biosynthèse de thionine chez l'Orge (Reinbothe et al., 1997), de protéines riche en proline ou de Vsp (Vegetative storage protéins) des graines de Soja (Stawick et al., 1991 ; Creelman and Al, 1992; Mason and Al, 1992, < RTI ID=0.0> 1993< /RTI> ; Shepherd and Al, 1995).

Lastly, it is also implied in the regulation of the protein biosynthesis taking part in the transduction of the signals in response to a stress, such as for example the lipoxygenases (Saravitz and Siedow, 1996) or even the systémine (Reinbothe and Al, 1994; Bergey and Al, 1996).

So it is not astonishing that various teams showed a reduction in the incidence of certain diseases following a treatment of the plant concerned with the vapors of MeJa (Cohen and Al, 1993; Meir and Al, 1998; Thomma and Al, 1998; Vijayan and Al, 1998; Thomma and Al, 2000). Moreover, J. S. Thaler (1999) shows that for many plants, various mechanisms of defence against the attack of herbivores are induced via the octadécanoïque way and would be thus implied in the attraction of natural enemies. For example, it shows that the treatment of seedlings by < RTI ID=0.0> AJ< /RTI> increase the parasitism of the caterpillars of vermin.

Chez la Vigne, les mécanismes de signalisation impliqués dans l'expression des réactions de défense ne sont pas encore bien connus. However, one finds the synthesis of the three types of molecules of defense (lignin, proteins of defense and phytoalexines). In particular, the role of phytoalexines is in particular held by a family of original compounds: polyphenols (Deloire and Al, 2000).

Present in more or less great quantities in all the bodies of the plant, the phytoalexines they are inductibles on the level of the sheets and bays. This type of induction is indicated under the term of elicitation. The factors of elicitation (or éliciteurs) can have different origins. It can be a question:

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< RTI ID=0.0> of biotic elicitation, < /RTI> for example at the time of an aggression by pathogenic like Botrytis cinerea, agent of the gray Rot (Jeandet and Al, 1995; Bavaresco and Al, 1997), Plasmopara viticola, agent of the Mildew < RTI ID=0.0> (Dercks< /RTI> and Creasy, 1989) or Phomopsis viticola, person in charge for Excoriose (Hoos and Blaich, 1990).

- of abiotic elicitation by the environmental factors such as U. V., temperature, light, asphyxiates, natural agents extracts of other plants (Jeandet and Al, 1997; Langcake and Pryce, 1977b; Douillet-Breuil et al., 1999), le chlorure d'aluminium (Adrian et al., 1996) ou l'ozone (Sarig et al., 1996).

At the time of a elicitation, phytoalexines like the transone, the trans picéide, the s-viniférine and the pterostilbene, can be induced on the level of the sheets and bays (Soleas and Al, 1997). This property of biosynthesis of novo of the phytoalexines in response to a stress, and in particular after attack by pathogenic, suggests that these molecules could play the part of natural means of defense of the plants.

This role of molecules of defense is corroborated by certain studies which seem to indicate a narrow correlation between the level of natural resistance of the plant and its aptitude to synthesize these molecules. For example, Langcake and I Carthy (1979) highlighted a relation between the resistance of certain species of the Vitis kind to Botrytis cinerea or Plasmopara viticola and their capacity of biosynthesis of phytoalexines (resvératrol and < RTI ID=0.0> viniférine). < /RTI> Moreover, Dercks and Creasy (1989) showed that the species resistant to Plasmopara viticola produce five times more phytoalexines than the significant species. In the same way, inside the Vinifera species, one finds type of vines more or less tolerant with the attack by mushrooms according to their output of phytoalexines.

The elicitation of the cells can be operated by means of agents or of various stresses, such as pressure, depression, empty, variation of pressure, presence of a gas, variable atmosphere, temperature, cold, light, cycle of luminosity, radiation,

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toxin, vegetable toxin, agitation, bacterium, virus, fungi, micro-organism, ultrasound, IR, UV, asphyxiate, etc

Any method of known elicitation of the specialist of the profession can be used to prepare a broyat usable in the composition according to the invention.

Thus, the broyats usable according to the invention can take any known form. One can, in particular, quote the broyats aqueous, alcoholic, in particular ethanolic or hydroalcoholic.

Preferentially according to the invention, the broyat is an aqueous broyat or a dry broyat or appreciably dryness.

Advantageously, the broyat can in a subsequent stage freeze-dried being.

According to an advantageous form, the broyat is a broyat cells in their culture medium or an extract of culture medium, the aforementioned broyat by after advantageously being dried or being freeze-dried.

The invention thus has as an aim a cosmetic composition containing at least a medium of différenciées vegetable cells, characterized in that the aforementioned medium is a broyat, différenciées vegetable cells, cultivated in an in vitro culture medium and élicitées in in vitro, the aforementioned culture medium broyat being dispersed in the aforementioned composition or being in a form ready to be dispersed in the aforementioned composition. The vegetable cells are preferentially cultivated in an in vitro culture medium and are preferentially élicitées in an in vitro culture medium to synthesize at least a phytoalexine, this elicitation being advantageously operated after a stage of in vitro culture of the vegetable cells without or appreciably without elicitation to synthesize at least a phytoalexine. the composition contains at least a broyat vegetable cells différenciées and élicitées in in vitro culture to synthesize at least a phytoalexine, this elicitation to synthesize at least a phytoalexine being advantageously operated after a stage of in vitro culture

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de cellules végétales sans élicitation, dans laquelle ledit broyat contenant au moins une phytoalexine comprend au moins 95%, avantageusement au moins 97%, de préférence au moins 99% en poids de l'ensemble des matières sèches issues des cellules végétales broyées dédifférenciées et élicitées in vitro, ledit broyat étant dispersé dans ladite composition ou étant sous une forme apte à être dispersée dans ladite composition.

In a preferred way, appreciably all, even all the whole of the matters dry resulting from the crushed dédifférenciées and élicitées vegetable cells in vitro.

If the cells are washed for eliminated any culture medium present, without affecting the membrane structure of the cells, it is possible to less obtain a broyat élicitées dédifférenciées cells not containing culture medium (for example than 0, < RTI ID=0.0> 1% < /RTI> in weight compared to the weight of the crushed cells).

Advantageously, the broyat of vegetable cells dédifférenciées and élicitées in vitro includes/understands particles coming from the vacuoles, of the particles coming from the cytoplasm and the particles coming from the membrane pecto cellulose, the aforementioned broyat containing at least 0,1% in weight of phytoalexine (S).

For example, the composition contains from 0,005 to 25% in weight, advantageously from 0,005 to 5% in weight of medium or broyat of dédifférenciées vegetable cells, this weight being calculated in dry form.

In an advantageous way, the composition contains a medium or broyat vegetable cells dédifférenciées and élicitées in vitro. Preferably, the broyat is a dry broyat or appreciably dryness of vegetable cells dédifférenciées and élicitées in vitro, the aforementioned culture medium broyat dry or appreciably dry containing a water content lower than 25% in weight, advantageously lower than < RTI ID=0.0> 15% < /RTI> in weight, preferably lower than 10% in weight. In particular, the aforementioned broyat dry or appreciably dryness contains a quantity of sufficient water to ensure the integrity of the membrane of the cells before the stage of crushing.

The broyat advantageously has an average granulometry of solid particles lower than < RTI ID=0.0> 100 < /RTI> < RTI ID=0.0> cm, < /RTI> advantageously lower than 10 < RTI ID=0.0> U. m, < /RTI> preferably

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inférieure à 1 < RTI ID=0.0> u. m. < /RTI> Advantageously, the particles of the broyat have a granulometry such as 90% in weight of the particles of crushed cells have a granulometry included/understood in the beach granulometry average-25% until average granulometry + 25%.

According to a particular embodiment, the aforementioned broyat of vegetable cells dédifférenciées and élicitées in vitro culture medium contains at least a phytoalexine synthesized by the elicitation of the vegetable cells dédifférenciées in in vitro culture medium, or a mixture of such phytoalexines.

According to an advantageous characteristic of an embodiment, the aforementioned broyat of dédifférenciées and élicitées vegetable cells is a broyat vegetable cells dédifférenciées and élicitées by an agent in in vitro, the aforementioned culture medium broyat being appreciably free of the aforesaid agent after elicitation in in vitro culture medium.

According to another advantageous characteristic of an embodiment, the aforementioned broyat of dédifférenciées and élicitées vegetable cells is a broyat vegetable cells dédifférenciées and élicitées by an agent in the culture medium, the aforementioned agent being an agent whose presence is wished in the cosmetic composition, such as for example, surface-active, a dispersing agent, an acid, etc

According to a preferred embodiment, the dédifférenciées cells are élicitées in in vitro culture medium by an agent bird, in particular a gas, such as < RTI ID=0.0> CO₂. < /RTI>

According to a preferred embodiment, the dédifférenciées cells are élicitées in in vitro culture medium by a physical agent, in particular the temperature, the light, electromagnetic fields, UV, the pressure, asphyxiation.

According to a detail of an embodiment, the aforementioned broyat of vegetable cells dédifférenciées and élicitées in in vitro culture medium contains at least one

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terpene or tannic or polyphenolic compound, the aforementioned compound being synthesized by in vitro elicitation of the vegetable cells dédifférenciées in their culture medium.

Advantageously, the broyat of vegetable cells dédifférenciées and élicitées in in vitro culture medium is appeared as a viscous suspension or a gel or an appreciably dry broyat, the aforementioned suspension, freezing or broyat being in a dispersible form in the composition.

According to a particular embodiment, the broyat of cells is a broyat cells of vine dédifférenciées and élicitées in in vitro culture medium.

According to a detail of a preferential embodiment, the composition contains a broyat cells dédifférenciées, cultivated and élicitées in their in vitro culture medium.

According to an advantageous embodiment, the composition contains a medium of cells dédifférenciées and élicitées in in vitro culture medium, in particular a broyat of dédifférenciées and élicitées cells, the aforementioned medium or broyat containing at least 0,1% in weight of stilbenes compared to the dry weight of the cells, in particular at least 0,2% in weight of stilbenes compared to the dry weight of the cells, preferably at least 0,5% in weight of stilbenes compared to the dry weight of the cells.

In the composition according to the invention, the broyat results from the culture of dédifférenciées vegetable cells, élicitées in in vitro culture medium then dried of the species Salvia, Coleus, Rosmarinus, Ginkgo, Cannabis, Colchicum.

Gloriosa, Asparagus, Argan, Glycine, Medicago, Mungo, Erythrina, Oenothera, Papaver, Atropa, Datura, Solanum, Borago, Reseda, Amsonia, Catharantus, Pilocarpus, Digitalis, Coffea, Theobroma, Jasminum, Capsicum, Iris, vine, taxus, sequoia, chlorophytum, Cocoa, psoralea corylifolia, vitex negundo, will commiphora wighii, eucalyptus punctata, lavandula angustifolia, citrus

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silt, vanilla planifolia, marrubium vulgare, pilocarpus jaborandi, pinks, betula, tea, and mixtures of cells of such species.

According to a possible embodiment, the composition according to the invention includes/understands différenciées vegetable cells crushed (for example with a granulometry lower than < RTI ID=0.0> 5jj. m) < /RTI> and of the vegetable cells différenciées élicitées not crushed or crushed more coarsely, vegetable cells différenciées élicitées not crushed or crushed more coarsely being identical or different with the crushed élicitées cells < RTI ID=0.0> et/ou < /RTI> presenting an elicitation different from the crushed élicitées cells. According to a particular embodiment, the différenciées vegetable cells are separate in two distinct fractions, a first being subjected to a fine crushing, while second is subjected to a coarse crushing or is not crushed.

The invention still has as an aim, a method of preparation of a composition with topic use according to the invention. In this process, one puts vegetable cells différenciées in an in vitro culture medium so as to allow to a growth of the cells, one elicit the aforementioned vegetable cells differentiated in their in vitro culture medium for one period of time sufficient for the synthesis from a sufficient quantity from metabolite (S), in particular of at least a secondary metabolite, preferably of at least a phytoalexine or a mixture of phytoalexine, and one mixes at least a medium or broyat of vegetable cells élicitées of the culture medium, with one or more excipients to prepare a composition with use topic, in particular cosmetic, for example for the treatment of the skin, the hair, leather, the nails, etc. The élicitées cells are crushed before and/or after their mixture with one or more excipients of the composition. Although it is possible to subject the cells différenciées and élicitées in culture medium in vitro to a crushing, it is advantageous to separate the cells différenciées and élicitées from the culture medium by not affecting appreciably the membranes of the cells and to subject the aforementioned cells then to a crushing, advantageously after one or

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stages of washing not affecting appreciably the membranes of the cells and/or after one or of the stages of drying.

According to a particular embodiment, the différenciées vegetable cells are subjected successively to stages of in vitro culture without elicitation and to stages of in vitro culture with elicitation.

In an advantageous way, one separates from the élicitées cells of in vitro culture medium, and one subjects the aforementioned extract to a drying, follow-up of a crushing.

Although it is possible to freeze-dry the différenciées cells élicitées separate and washed without affecting the structure of the membranes of the cells and to crush the lyophilisat then, it is advantageous to subject the différenciées cells élicitées separate and washed without affecting the structure of the membranes of the cells, with a drying controlled so as not to affect the membrane barrier appreciably (temperature of drying of less < RTI ID=0.0> 50 C, < /RTI> water content of the cells from at least 3%, for example of 5 with < RTI ID=0.0> 15%), < /RTI> and then with a crushing. This makes it possible to break the cells (membranes, cytoplasm and vacuoles) at the time of the stage of crushing, so as to ensure the release of the phytoalexines at the time of this stage.

The stage of crushing and/or the stage of drying (controlled in particular) and/or the stage of washing and/or the stage of separation of the différenciées cells are advantageously carried out in the presence of one or several antioxydant agents, such as vitamin E, etc, to avoid or reduce any oxidation of one or several compounds of the cells (for example of the membrane).

Preferably, one elicit cells in their in vitro culture medium by means of an agent, which after extraction of the élicitées cells does not find in the broyat élicitées cells.

Advantageously, in the process according to the invention, one controls the stage of elicitation of the aforesaid vegetable cells differentiated in their culture medium

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in vitro, so as to obtain a medium containing at least 0,1% in weight of stilbenes compared to the dry weight of the différenciées cells, in particular at least 0,2% in weight of stilbenes compared to the dry weight of the cells, preferably at least 0,5% in weight of stilbenes compared to the dry weight of the cells.

The medium or broyat results for example from the culture of différenciées vegetable cells, élicitées in in vitro culture medium, then advantageously dried, of the species Salvia, Coleus, Rosmarinus, Ginkgo, Cannabis, Colchicum, Gloriosa, Asparagus, Arganier, Glycine, Medicago, Mungo, Erythrina, Oenothera, Papaver, Atropa, Datura, Solanum, Borago, Reseda, Amsonia, Catharantus, Pilocarpus, Digitalis, Coffea, Theobroma, Jasminum, Capsicum, Iris, vine, taxus, sequoia, chlorophytum, Cacao, psoralea corylifolia, vitex negundo, will commiphora wighii, eucalyptus punctata, lavandula angustifolia, citrus silt, vanilla planifolia, marrubium vulgare, pilocarpus jaborandi, pinks, betula, tea, and mixtures of cells of such species.

An example of preparation of broyat usable according to the invention is given in addition in the examples.

The quantity of broyat present in the composition according to the invention is of course a function of the required effect and can thus vary on the whole.

To give an order of magnitude, one can previously use a broyat such as definite in a quantity representing of 0, < RTI ID=0.0> 01% < /RTI> to 20% of the total weight of the composition and preferentially in a quantity representing from 0,1% to 5% of the total weight of the composition.

The broyat of vegetable cells différenciées and élicitées in in vitro culture medium, in particular to support the

production of one or several phytoalexines, for example is used as an antioxydant agent, radicalizing anti agent, alleviating agent, agent anti-irritant, an agent scavenger free radicals.

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In a particular aspect the process of the invention allows obtaining a broyat enriched in flavonoïdes the such flavanols, the anthocyanes and flavonols.

In another particular aspect the process of the invention allows obtaining a broyat enriched into made up not flavonoïdes the such acids phenols, derived from the benzoic acid but also from the original compounds like stilbenes, isomers trans- and cis- of the resvératrol and their glucosides, them trans- and cis- < RTI ID=0.0> picéïdes. < /RTI>

In another particular aspect of the invention the broyat enriched out of polyphenols, phytoalexines and metabolites secondary would retain their important pharmacological effects. Thus, the broyats of the invention present one or more activities chosen among antioxydant, anti-radicalizing, anti-inflammatoire, anti-proliferative, releasing, vascular, ? etc

Contrary to the primary metabolites, the secondary metabolites such as polyphenols accumulate in the plant in vivo in small quantity. In an aspect of the invention in order to obtain these secondary metabolites in large and and stable quantity continuously, we stimulated their biosynthesis in cultures of standardized cells.

The vegetable cells différenciées usable in the composition according to the invention can come from all the known species of plants.

In this respect one will quote the kinds Salvia, Coleus, Rosmarinus, Ginkgo, Cannabis, Colchicum, Gloriosa, Asparagus, Glycine, Medicago, Mungo, Erythrina, Oenothera, Papaver, Atropa, Datura, Solanum, Borago, Reseda, Amsonia, Catharantus, Pilocarpus, Digitalis, Coffea, Theobroma, Jasminum, Capsicum, Iris, vine, taxus, sequoia, chlorophytum, Cacao, psoralea corylifolia, vitex negundo, will commiphora wighii, eucalyptus punctata, lavandula angustifolia, citrus silt, vanilla planifolia, marrubium vulgare, pilocarpus jaborandi, pinks, betula, tea, etc

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Particularly according to the invention one uses différenciées vegetable cells coming from plants from the sequoia kinds, vine, argan, cocoa and chlorophytum, and of the combinations of those.

Of course the broyats of vegetable cells différenciées usable in the composition according to the invention can come from mixtures of différenciées vegetable cells obtained starting from different vegetable kinds and/or obtained starting from different vegetable material, the aforementioned cells being élicitées in the same in vitro culture medium or in culture media in vitro different (for example to operate different élicitations).

By composition with topic use one understands creams, ointments, lotions, suspensions, sticks, shampoos, gel, solutions (for example applicable by spray). The composition with topic use is for example a composition cosmetic, dermatological, a composition of hygiene of the skin, a perfume, etc

Preferentially according to the invention, the composition is a cosmetic composition, particularly of topics application.

The present invention has moreover as an aim a cosmetic process of treatment of the skin, characterized by the fact that < RTI ID=0.0> one < /RTI> apply to the skin, the hair, and/or the mucous membranes, a composition according to the invention including/understanding at least a broyat vegetable cells différenciées and élicitées in culture medium in vitro, freeze-dried and incorporated or dispersed in a cosmetic product.

The cosmetic process of treatment of the invention can be implemented in particular by applying the cosmetic compositions such as above definite, according to the technique of usual use of these compositions. By

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example: application of creams, gel, serums, lotions, milks, shampoos or anti-solar compositions on the skin.

The essential characteristics of compositions according to the invention, processes according to the invention, and broyats according to the invention are given in the claims. The following examples and compositions illustrate the invention without limiting it at all. In the compositions the proportions indicated are percentages in weight.

In these examples, one used a process preferred such as definite in short ciaprès.

Proceeded of obtaining one crushed.

Stage 1: Preparation of cells différenciées and cultivated in an in vitro culture medium Stage 2 < RTI ID=0.0> : Elicitation < /RTI> cells différenciées in the culture medium Stage 3: Extraction of the cells différenciées and élicitées in culture medium in vitro, for example by filtration of the culture medium followed by one or several stages of washing, operated in particular not to destroy the structure of the membranes of the cells. The cells are advantageously washed to appreciably eliminate any trace from medium D culture, this washing being operated so as not to destroy the structure of the membrane of the cells.

Stage 4: Drying or freeze-drying of the cells différenciées and élicitées in in vitro culture medium, this operation of drying being advantageously operated not to destroy the structure of the membranes of the cells.

This stage is advantageously operated at a temperature lower than < RTI ID=0.0> 60 C, < /RTI> for example < RTI ID=0.0> between -60 C < /RTI> and < RTI ID=0.0> 50 C. < /RTI>

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Stage 5: crushing (stage 5 is advantageously operated, however in certain cases, this stage can prove nonnecessary). The broyat contains all the dry components thus appreciably forming the cell, namely appreciably all the component of the membrane, the cytoplasm and the vacuoles.

Stage 6: mix and/or incorporation with excipients and/or other active ingredients (in particular of other vegetable

cells/broyats) for the preparation of the composition to use topic Exemple 1 Realization of a < RTI ID=0.0> broyât< /RTI> cells différenciées and élicitées in vitro of vine.

The first stage for the development of vegetable cellular cultures consists in selecting the plant producing the required substances. One admits today that within the same species, there is a variability of the outputs of a metabolite given, partly of genetic origin.

When that is possible, it will thus be necessary to exploit this variability by selecting the best genotype, i.e. most productive for the metabolite required. From aseptized fragments of a body of selected plant (sheet, stem, root?), placed in vitro on a solid medium (gélosé), one manages to induce primary proliferations. Thus, after a few weeks of setting in culture, it is formed, on the level of explants, of the undifferentiated cellular clusters, called cal. The growth of these cal will be maintained by successive road repairs on a new nutritive medium. The conditions of culture then will induce the spontaneous appearance of a morphological and metabolic variability between cal resulting < RTI ID=0.0> of une< /RTI> even plant or of the same explant. However, the maintenance of constant environmental conditions tends to decrease this variability. Thus, after one to two years of regular road repairs, one obtains a collection of stable stocks presenting of the characteristics of growth and production of very different metabolites.

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This stage, it is then possible to select, using well defined tests, the stocks producing the compounds of interest in large quantity. The passage of these cal in liquid medium then makes it possible to be directed towards more important volumes of production, first of all out of flasks of 250 ml, and later out of bio-engine (20 Liters and more). The cellular suspensions thus obtained, formed of aggregates and insulated cells, can still have a heterogeneity (variability < RTI ID=0.0> somaclonale). < /RTI> An additional selection is then carried out to obtain highly producing cellular lines. In complement of this cloning, the production of the metabolite of interest can also be optimized by the modification of the conditions of culture leading to development of mediums, said mediums of production. This medium is identical in the middle of maintenance of the cells except for the saccharose concentration which is multiplied by two. During their culture in a medium of production, the highly producing cellular lines of Vine type of vine Cabernet Sauvignon are élicitées ten days after the inoculation, by light UV 254 Nm using a lamp < RTI ID=0.0> Wilber-Lourmat< /RTI> T-30C (600 < RTI ID=0.0> I/W/m²), < /RTI> setting at a distance from lm in direct lighting above the cells during 10 minutes, which induces a considerable accumulation of polyphenols, in particular of stilbenes, in the cells. This means of elicitation does not form obviously any impurity in the cellular culture. At the end of the culture, i.e. three days after the elicitation, the vegetable cells are filtered to eliminate the remaining culture medium and are rinsed with cold water < RTI ID=0.0> (4 C).< /RTI> One thus obtains a fresh biomass of approximately 350 grams per liter of culture the extracted cells is then dried and crushed. A more or less important drying is carried out in order to obtain the broyat cells of Vine différenciées and élicitées in vitro, in the form of a viscous suspension or of a gel or an appreciably dry powder. By controlling the speed of drying and the water content of the cells, advantageously between 3 < RTI ID=0.0> and 10%, < /RTI> it is possible not to destroy the membrane of the cells before the stage of crushing, this allows that the phytoalexines contained in the cells, in particular in the vacuoles of the cells < RTI ID=0.0> et/ou< /RTI> in the membrane, are released at the time of the stage of crushing. In the case of cells appreciably dry, one obtains after freeze-drying using one

< Desc/Clms Page number 27>

Virtis apparatus (Plain-Trap < RTI ID=0.0> 10-100) < /RTI> approximately 20 grams of biomass dries per liter of culture. The powder obtained after crushing using a standard crusher Mortar, is light, ultrafine (size of the particles lower than 10 < RTI ID=0.0> lm) < /RTI> and of color beige-clearly.

The élicitées and dried cells are crushed to form particles having an average granulometry (in weight) ranging between 1 and < RTI ID=0.0> 101µ.< /RTI> It is possible to subject if necessary crushing it to a separation granulometric (sieve, etc) to preserve only particles of a beach of determined granulometry, for example of the beach < RTI ID=0.0> 5-1< /RTI> < RTI ID=0.0> m, < /RTI> < RTI ID=0.0> 1-5< /RTI> < RTI ID=0.0> U. m, < /RTI> less than 3 m, etc

The différenciées cells were prepared starting from various matters and were élicitées by means of various agents. These data are included in the following table:
EMI27.1

< tb>	< example; SEP> 1 < SEP> (vine) < SEP> matter < SEP> of which < SEP> < SEP> cells < SEP> < type; SEP> of elicitation
< tb>	< SEP> différenciées
< tb>	< SEP> come
< tb>	1A < SEP> branch < SEP> of < SEP> less < SEP> of a < SEP> year < SEP> ray < SEP> UV < SEP> during < SEP> 3
< tb>	< SEP> of age < SEP> days
< tb>	1B < SEP> cuticule < SEP> of < SEP> grape < SEP> ripe < SEP> gas < SEP> carbonic < SEP> during
< tb>	< SEP> 24 < SEP> hours
< tb>	1C < SEP> cuticule < SEP> of < SEP> grape < SEP> green < SEP> gas < SEP> carbonic < SEP> during < SEP> 2
< tb>	< SEP> days

< tb>
 < tb> 1D < SEP> pip < SEP> of < SEP> grape < SEP> ray < SEP> UV < SEP> and < SEP> gas
 < tb>
 < tb> < SEP> carbonic < SEP> during < SEP> 5
 < tb>
 < tb> < SEP> days
 < tb>
 < tb> 1E < SEP> < root; SEP> ray < SEP> UV < SEP> during < SEP> 12
 < tb>
 < tb> < SEP> hours
 < tb>
 < Desc/Cllms Page number 28>

EMI28.1

< tb>
 < tb> 1F < SEP> break into leaf < SEP> green < SEP> ray < SEP> UV < SEP> during < SEP> 15
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> hours
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> 1G < SEP> bud < SEP> ray < SEP> UV < SEP> during < SEP> 75
 < tb>
 < tb>
 < tb> < SEP> hours
 < tb>
 < tb>
 < tb>
 < tb> 1H < SEP> residue < SEP> of a < SEP> stage < SEP> of < SEP> ray < SEP> UV < SEP> during < SEP> 2 < SEP> days
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> pressing
 < tb>
 < tb>
 < tb>
 < tb> it < SEP> residue < SEP> of a < SEP> stage < SEP> of < SEP> ray < SEP> UV < SEP> during < SEP> 3 < SEP> days
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> pressing
 < tb>

One will note moreover than it is interesting to carry out the operation of crushing of the cells différenciées and élicitées in the presence of one or several agents or excipients of the cosmetic composition so as to ensure the release of the phytoalexines in at least certain agents of the cosmetic composition.

Example 2 Determination by HPLC of the content stilbenes in élicitées cells of vine (< example; RTI ID=0.0> 1A) < /RTI> and élicitées.

Materials and methods: - Pump Bischoff Model 2.200 - automatic Injector Alcotec Model 788 autosampler - Column Ultrasep < RTI ID=0.0> C 18< /RTI> (30 cm X 0, 18 cm) 6 mm of porosity - Detector of fluorescence, Jasco 821-FI
 The detection of fluorescence was carried out with an excitation with 300 Nm and an emission with 390nm. Éluant used is composed of methanol: water, 40: 60 (v/v) whose pH 8,3 was adjusted with KOH 1M.

Result: The content stilbenes is approximately 1% compared to the dry matter weight of élicitées cells of Vine in vitro. The content of phytoalexines remains lower than 0,05% in the not élicitées cells. This high percentage of stilbene (20 times more) is a method of control of the stage of elicitation, and thus of the manufacturing process of a broyat according to the invention. Considering the concentration

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important in stilbene in the broyat according to the invention, it is possible by processes of extraction, distillation, crystallisation, etc to produce mediums more concentrated even in stilbenes or phytoalexines, to even produce appreciably pure photoalexines.

The compositions according to the invention will thus present a report/ratio content of phytoalexines/content of cellular membrane crushed important compared to the phytoalexines ratio/content of cellular membrane crushed for not élicitées and/or natural vegetable cells.

Pharmacological example 3 Activity of a < RTI ID=0.0> broyat< /RTI> of vine: antioxydant the anti-ridicalizing activity of the product obtained according to the < example; RTI ID=0.0> 1A< /RTI> was studied in vitro. We used a model of reconstituted skins < RTI ID=0.0> SKINETHIC (G), < /RTI> allowing to reveal this activity by proportioning of malondialdéhyde (MDA), after its induction by ultraviolet rays B.

Distribution of the skins the test was led in triplicate, after 24 hours of contact of the product (broyat of the 1A example) with the skins. Kératinocytes of human origin are sown on polycarbonate filters of 0,63 cm² in a definite medium (MCDB 153 modified) and supplemented. The cells are cultivated during 14 days with the interface air/liquid, the culture medium being changed every two days.

The skins thus formed were used for the realization of the study as from the 17th day of the culture.

Batches of reconstituted skins < RTI ID=0.0> SKINETHICO< /RTI> :

Batch 1 < RTI ID=0.0>: 3 épidermes< /RTI> witnesses not receiving neither produced nor irradiation

Batch 2 3 skins irradiated with the UVB (150 < RTI ID=0.0> mJ/cm²) < /RTI>

Batch 3 < RTI ID=0.0>: 3 épidermes< /RTI> treaties with SOD+Catalase + UVB (150 < RTI ID=0.0> J/cm²) < /RTI>

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- Batch 4 3 skins treated with broyat (0, < RTI ID=0.0> 1%) < /RTI> - Batch 5 < RTI ID=0.0>: 3 épidermes< /RTI> treaties with broyat (0,5%) - Batch 6 3 skins treated with broyat (1%) - Batch 7 < RTI ID=0.0>: 3 skins traités< /RTI> with broyat (0, < RTI ID=0.0> 1%) < /RTI> + UVB < RTI ID=0.0> (150 mJ/cm²) < /RTI> < RTI ID=0.0> - Batch 8: < /RTI> 3 skins treated with broyat (0,5%) + UVB < RTI ID=0.0> (150 mJ/cm²) < /RTI> < RTI ID=0.0> - Lot< /RTI> 9 : 3 skins treated with broyat (1%) + UVB (150 mJ/cm²) Proportioning of malondialdéhyde (MDA): index of lipoperoxydation Extraction of the malondialdéhyde < RTI ID=0.0> (MDA) < /RTI> 24 hours after the treatment of the reconstituted skins < RTI ID=0.0> SKINETHIC#, < /RTI> the cellular ones were suspended in: - 250 L of plug Sorting 50 mm, pH 8 container < RTI ID=0.0> NaCl< /RTI> 0, 1M; EDTA 20 mm < RTI ID=0.0> - 25 tl< /RTI> SDS with 7% - 300 L of HCl (0, 1 NR) - 38 L of acid phosphotungstic with 1% in water - 300 L of acid thiobarbituric with 0,67% in water After 1 hour of incubation in the darkness with < RTI ID=0.0> 50 C< /RTI> and a cooling in frozen water, 300 N-butanol L were added in each tube. Those were centrifuged to 10.000 G with < RTI ID=0.0> 0 C< /RTI> during 10 minutes. The higher phase was recovered for the proportioning of the MDA.

Proportioning of malondialdéhyde (MDA) the MDA was proportioned by measurement of fluorescence after separation of the complex < RTI ID=0.0> MDA-TBA by HPLC.< /RTI>

- Pump Bischoff Model 2.200 - automatic Injector Alcot Model 788 autosampler

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- Column Ultrasep C18 (30 cm X 0,18 cm) 6 mm of porosity - Detector of fluorescence, Jasco 821-FI the detection of fluorescence was carried out with an excitation with 515 Nm and an emission with 553nm. Éluant used is composed of methanol: water, 40: 60 (v/v) whose pH 8,3 was adjusted with KOH < RTI ID=0.0> 1M.< /RTI>

The quantification was made compared to standards treated like the samples (0,125; 0,25 ; 0,5 and 1 uM) using a data-processing software ICS (Peak 3) (Consumable Instrumentation Service).

Proportioning of proteins the proportioning of proteins was carried out according to the method of < RTI ID=0.0> BRADFORD.< /RTI>

The increase in the absorbance with 595 Nm is proportional to the concentration of proteins determined using a spectrophotometer UNICAM 8625.

Résultats Dosage of malondialdéhyde (MDA) in the cellular homogenate the results is gathered in the table below: EMI31.1

< tb>
 < tb> < SEP> MDA < SEP> (pM/mg < SEP> proteins) < SEP> Variation < SEP> of
 < tb>
 < tb>
 < tb>
 < tb> < SEP> MDA < SEP> (in < SEP> %) < SEP> by
 < tb>
 < tb>
 < tb>
 < tb> < SEP> < report/ratio; SEP> with
 < tb>
 < tb>
 < tb>
 < tb> < SEP> witness
 < tb>
 < tb>
 < tb>
 < tb> Witness < SEP> 546 < SEP> + < SEP> 40, < SEP> 81
 < tb>
 < tb>

< tb>
 < tb> broyat < SEP> (0, < SEP> 1%) < SEP> 445,3 < SEP> 57,72*-18 < SEP> (reduction)
 < tb>
 < tb>
 < tb> broyat < SEP> (0,5%) < SEP> 409,5 < SEP> 48, < SEP> 58*-25 < SEP> (reduction)
 < tb>
 < tb>
 < tb>
 < tb> broyat < SEP> (1%) < SEP> 325,5 < SEP> 28, < SEP> 85*-40 < SEP> (reduction)
 < tb>

< Desc/Cims Page number 32>

* Significantly different compared to the witness: $p < 0,005$ (Wilcoxon Rank Sum Test).

The results obtained reveal a significant protection of the broyat with respect to the physiological lipoperoxydation with dilutions 0,1; 0,5 and 1% respectively of 18%, 25% and 40%.

< RTI ID=0.0> Lipopéroxydation provoquée< /RTI> The results are gathered in the table below:
EMI32.1

< tb>
 < tb> < SEP> MDA < SEP> Variation < SEP> of < SEP> MDA
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> (pM/mg < SEP> proteins) < SEP> (in < SEP> %) < SEP> by < SEP> report/ratio
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> with the < SEP> witness < SEP> with
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> UVB
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Witness < SEP> 546 < SEP> ~ < SEP> 40,81 < SEP> -
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> UVB < SEP> (150 < SEP> mJ/cm) < SEP> 792,4 < SEP> 59,4 < SEP> (increase < SEP> of
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> 45% < SEP> by < SEP> < report/ratio; SEP> with
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> witness < SEP> without < SEP> UVB)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> SOD/Catalase < SEP> + < SEP> UVB < SEP> 482,5 < SEP> ~ < SEP> 22, < SEP> 1-39 < SEP> (reduction)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> (150 < SEP> mJ/cm2)
 < tb>
 < tb>

< tb>
 < tb>
 < tb>
 < tb> broyat < SEP> (0, < SEP> 1%) +UVB < SEP> 545 < SEP> ~ < SEP> 43, < SEP> 4 ** - 31 < SEP> (reduction)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> (150 < SEP> mJ/cm2)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> broyat < SEP> (0,5%) < SEP> + < SEP> UVB485, < SEP> 535, < SEP> 6 ** - 39 < SEP> (reduction)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> (150 < SEP> mJ/cm2)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> broyat < SEP> (1%) < SEP> + < SEP> UVB < SEP> 420, < SEP> 3 < SEP> 46, < SEP> 3*-47 < SEP>
 (reduction)
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> (150 < SEP> mJ/cm2)
 < tb>

* Significantly different compared to the witness p < 0,005 (Wilcoxon Rank Sum Test).

< Desc/Clms Page number 33>

** Significantly different compared to the witness p < RTI ID=0.0> < < /RTI> 0,05 (Wilcoxon Rank Sum Test).

The results obtained reveal a significant protection of the broyat with respect to the lipoperoxydation caused by the ultraviolet rays B (150 < RTI ID=0.0> mJ/cm2) < /RTI> with the concentrations of 0,1; 0,5 and 1% respectively of 31%, 39% and 47% compared to the protective enzymes SOD/catalase (39%).

Under the experimental conditions selected, the broyat appeared to present a significant anti-ridicalizing activity at the level of the reconstituted skins < RTI ID=0.0> SKINETHICO < /RTI> after 24 hours of contact. This activity was revealed by the proportioning of the MDA.

Indeed, proportionings of the MDA show that the broyat studied with the concentrations of < RTI ID=0.0> 0, < /RTI> 1; 0, < RTI ID=0.0> 5etl% < /RTI> : - the rate of the physiological MDA respectively of < decreases significantly; RTI ID=0.0> 18%, < /RTI> 25% and 40%.

- significantly protects the cells against the lipoperoxydation caused by the ultraviolet rays B (UVB 150 < RTI ID=0.0> mJ/cm2) < /RTI> by decreasing the rate of induced MDA of 31%, 39% and 47% compared to the SOD-catalase which decreases the rate of MDA by 39%.

In conclusion, the broyat as well presents a anti-ridicalizing effect under physiological conditions as under the conditions of induction by the ultraviolet radiations B. It comes out from this test that the broyat presents a significant anti-ridicalizing effect.

Taking into account the model retained (reconstituted skin), it can right now be planned to use broyat in preparations with topics use with the minimal amount activates of 0, < RTI ID=0.0> 1%. < /RTI>

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Example 4 Research of the effect of the product on the speed of breathing (oxygen uptake in oxygen nanoatome per million cells and minute)

This experiment was carried out according to 2 different conditions: - Effect on the speed of basal breathing cellular on the level of the not permeabilized cells and in the presence of glucose to evaluate cellular breathing.

- Effect on the speed of breathing of the cells permeabilized in the presence of the respiratory substrate, pyruvate-malate, to evaluate breathing mitochondriale.

This study was carried out on human k ratinocytes in culture dissociated with trypsin. 5 to 10 million k ratinocytes in culture were put in suspension in 1 < RTI ID=0.0> ml < /RTI> of Hanks-Hepes medium to < RTI ID=0.0> 30 C < /RTI> container of glucose (20 < RTI ID=0.0> mm). < /RTI> Breathing was followed in real times and was expressed in oxygen nanoatomos spent per minute and 106 cells. The addition of various quantities of the product in the tank of the oxygraphe made it possible to highlight a possible stimulation or inhibition of breathing.

The quantity of oxygen dissolved in a medium of incubation was given using an electrode of Clark. The oxygen which diffuses through a Teflon film is tiny room to the level of the polarized cathode of turntable in-0.8 Volt. Under these

conditions the power being on between this cathode and the money anode are proportional to the oxygen concentration in the solution. The ionic bridge is ensured by a saturated half solution of KCl. The acquisition and the treatment of measurements are made on a microcomputer.

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- Effect on the speed of cellular basal breathing

This test was carried out on whole cells not permeabilized in the presence of glucose.

The tests were carried out starting from a solution mother of the product with < RTI ID=0.0> 0, 3%.< /RTI>

The results are gathered in the table below:

Speed of cellular basal breathing

EMI35.1

< tb>

< tb>

< tb> broyat

< tb>

< tb>

< tb>

< tb>

< tb> (pLideproduit) < SEP> 0.00 < SEP> 2 < SEP> 5 < SEP> 10 < SEP> 50 < SEP> 100

< tb>

< tb>

< tb>

< tb>

< tb>

< tb> Breathing

< tb>

< tb>

< tb>

< tb>

< tb>

< tb> basal < SEP> 1, < SEP> 15 < SEP> ~ < SEP> 1, < SEP> 22~ < SEP> 0, < SEP> 21 < SEP> 1, < SEP> 31 & < SEP> 0, < SEP> 14 < SEP> 1, < SEP> 58 < SEP> 2, < SEP> 30 < SEP> 2, < SEP> 58

< tb>

< tb>

< tb>

< tb>

< tb> (natomes/minute/0,5 < SEP> 0,64 < SEP> 0,27 < SEP> 0,21

< tb>

< tb>

< tb>

< tb>

< tb>

< tb> 106 < SEP> concealment.) < SEP> (n=3)

< tb>

< tb>

< tb>

< tb>

< tb> % < SEP> of < SEP>

< tb>

< tb>

< tb>

< tb>

< tb>

< tb> breathing < SEP> 100 < SEP> 106 < SEP> 114 < SEP> 137 < SEP> 200 < SEP> 224

< tb>

< tb>

< tb>

< tb>

< tb> cellular < SEP> basal

< tb>

- Effect on the speed of breathing mitochondriale

This test was carried out on the cells permeabilized in the presence of the respiratory substrate, pyruvate-malate.

The results are gathered in the tables below.

Speed of breathing mitochondriale in the presence of the pyruvate-malate.

EMI35.2

< tb>

< tb>

< SEP> Broyat

< tb>

< tb>
 < tb> < SEP> (, < SEP> ul < SEP> of < SEP> product) < SEP> 0.00 < SEP> 2 < SEP> 5 < SEP> 10 < SEP> 50 < SEP> 100
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Breathing
 < tb>
 < tb>
 < tb> < SEP> pyruvate < SEP> 1, < SEP> 5 < SEP> 1,98~ < SEP> 2,05~ < SEP> 2,25~ < SEP> 2, < SEP> 58 < SEP> 3, < SEP> 02
 < tb>
 < tb>
 < tb> (natomes/minute/0, < SEP> 12 < SEP> 0,05 < SEP> 0,14 < SEP> 0,42 < SEP> 0,22 < SEP> 0, < SEP> 38
 < tb>
 < tb>
 < tb> < SEP> 106 < SEP> concealment. < SEP> n=3
 < tb>
 < tb>
 < tb>
 < tb> < SEP> % < SEP> of < SEP>
 < tb>
 < tb>
 < tb> < SEP> breathing < SEP> 100 < SEP> 125 < SEP> 130 < SEP> 142 < SEP> 163 < SEP> 191
 < tb>
 < tb>
 < tb> < SEP> pyruvate
 < tb>
 < Desc/Clms Page number 36>

The results show as the broyat, with various amounts, as well increases the speed of breathing (oxygen uptake) on the level of the not permeabilized whole cells (in the presence of glucose), representing an increase in cellular basal breathing, that on the level of the permeabilized cells (in the presence of the pyruvate-malate), representing an increase in breathing mitochondriale.

Example 5 Research of the effect of the product on the speed of synthesis of ATP in nmoles per million of cells and minute.

This stage was carried out according to 2 different conditions: - Effect on the speed of synthesis of ATP on the level of the not permeabilized cells and in the presence of glucose to evaluate the speed of synthesis cellular.

- Effect on the speed of synthesis of ATP on the level of the cells permeabilized in the presence of the respiratory substrate, pyruvate-malate, to evaluate the speed of synthesis mitochondriale.

This study was carried out on human k ratinocytes in culture dissociated with trypsin. 5 to 10 million k ratinocytes in culture are put in suspension in 1 ml of Hanks-Hepes medium at < RTI ID=0.0> 30 C< /RTI> container of glucose (20 mm). The addition of various quantities of the product in the tank made it possible to highlight a possible activation or inhibition the speed of synthesis of ATP.

The quantity of ATP present in the medium was given thanks to the following enzymatic reaction:
EMI36.1

Lucif rase ATP + LUCIFERIN 'OXYLUCIFERINE + AMP + pi +CO2 + hv
Mg2+

< Desc/Clms Page number 37>

It is carried out in an apparatus of the < type; RTI ID=0.0> Luminoscan< /RTI> by using the ATP monitoring reagent (ATP Bioluminescence Assay Kit HS II) of Boehringer Mannheim.

The intensity of the light emitted during this reaction was measured by a < RTI ID=0.0> luminom tre< /RTI> (Luminoscan) which transcribes it in RLU (relative unit of luminosity).

The measured RLU were converted into mole of ATP while referring to a range standard of ATP.

- Effect on the speed of synthesis of ATP: rate of basal cellular synthesis

This test was carried out on whole cells not permeabilized in the presence of glucose. The results are gathered in the table below.

Speed of synthesis of ATP
EMI37.1

< tb> BROYAT
 < tb> (pl < SEP> of < SEP> product) < SEP> 0.00 < SEP> 2 < SEP> 5 < SEP> 10 < SEP> 50 < SEP> 100
 < tb>

< tb> Speed < SEP> of < SEP> synthesis
 < tb> (nmolesATP/mn/106 < SEP> concealment.) < SEP> 2, < SEP> 58 < SEP> 2, < SEP> 65 < SEP> 3, < SEP> 08 < SEP> 3, < SEP> 12~ < SEP> 5, < SEP> 80 < SEP> 5, < SEP> 95~
 < tb> (n=3) < SEP> 0,15 < SEP> 0,12 < SEP> 0,22 < SEP> 0,30 < SEP> 0,95 < SEP> 0,72
 < tb>
 < tb> % < SEP> of < SEP> synthesis < SEP> cellular
 < tb> < SEP> 100 < SEP> 103 < SEP> 119 < SEP> 121 < SEP> 225 < SEP> 231
 < tb>
 - Effect on the speed of svnthèse of ATP mitochondriale
 Speed of synthesis of ATP mitochondriale in the presence of the pyruvate-malate.
 EMI37.2

< tb>
 < SEP> BROYAT
 < tb> < SEP> (. < SEP> 1 < SEP> of < SEP> product) < SEP> 0.00 < SEP> 2 < SEP> 5 < SEP> 10 < SEP> 50 < SEP> 100
 < tb>
 < tb> Speed < SEP> of < SEP> synthesis
 < tb> (nmolesATP/mn/106 < SEP> 4, < SEP> 15 < SEP> 4, < SEP> 95~ < SEP> 4, < SEP> 95 < SEP> 5, < SEP> 02 < SEP> 5, < SEP> 75~ < SEP> 7, < SEP> 58
 < tb> < SEP> concealment. < SEP>) < SEP> (n=3) < SEP> 0,22 < SEP> 0,16 < SEP> 0,18 < SEP> 0,16 < SEP> 0, < SEP> 28 < SEP> 0,85
 < tb>
 < tb> < SEP> % < SEP> of < SEP> synthesis
 < tb> < SEP> mitochondriale < SEP> 100 < SEP> 119 < SEP> 119 < SEP> 121 < SEP> 139 < SEP> 183
 < tb>
 < Desc/Clms Page number 38>

The results show as the broyat, with various amounts, as well.increases the speed of synthesis of ATP on the level of the not permeabilized whole cells (in the presence of glucose), representing an increase in the synthesis of cellular ATP, that at the level of the permeabilized cells (in the presence of the pyruvate-malate) representing an increase in the synthesis mitochondriale.

Example 6 Research of the effect of the product on the energy metabolism of the cells in culture. Proportioning of cellular nucleotides adenylic < RTI ID=0.0> (ATP, < /RTI> ADP and AMP) in < RTI ID=0.0> protein nmoles/mg, < /RTI> and calculation of the energy load (EC) of the treated cells 5 days by the product.

The human k ratinocytes were put in culture during 5 days in absence and in the presence of the product < RTI ID=0.0> (107< /RTI> cells by measurement).

Once trypsinized, the cells were collected and the adenylic concentrations of nucleotides were determined by HPLC.

This test was carried out on not permeabilized whole cells.

The results are gathered in the table below.

EMI38.1

< tb>
 < tb>
 < tb>
 < SEP> Amounts < SEP> [ATP] < SEP> [ADP] < SEP> [AMP] < SEP> [ATP/ADP] < SEP> Summon < SEP> C. < SEP> E.
 < tb>
 < tb>
 < tb> control < SEP> 4222 < SEP> 1014 < SEP> 748 < SEP> 4.16 < SEP> 5984 < SEP> 0. < SEP> 79
 < tb>
 < tb>
 < tb> < SEP> 0,02% < SEP> 4532 < SEP> 1435 < SEP> 837 < SEP> 3.16 < SEP> 6804 < SEP> 0.77
 < tb>
 < tb>
 < tb> < SEP> 0,05% < SEP> 5292 < SEP> 1327 < SEP> 779 < SEP> 3.99 < SEP> 7398 < SEP> 0.80
 < tb>
 < tb>
 < tb> < SEP> 0,1% < SEP> 6184 < SEP> 1231 < SEP> 796 < SEP> 5. < SEP> 02 < SEP> 8211 < SEP> 0. < SEP> 83
 < tb>
 < tb>
 < tb> < SEP> 0, < SEP> 5% < SEP> 6848 < SEP> 2195 < SEP> 978 < SEP> 3. < SEP> 12 < SEP> 10021 < SEP> 0.79
 < tb>
 < tb>
 < tb> < SEP> 1% < SEP> 7791 < SEP> 2532 < SEP> 1131 < SEP> 3. < SEP> 08 < SEP> 11454 < SEP> 0. < SEP> 79
 < tb>
 The concentrations in ATP, ADP and AMP are expressed in nmoles/mg proteins (n=3).

< RTI ID=0.0> Somme= [ATP] < /RTI> + [ADP] + [AMP] energy Load (C. E.) < RTI ID=0.0> =< /RTI> [ATP] + 1/2 < RTI ID=0.0> [ADP]/([ATP] < /RTI> + [ADP] + [AMP])

< Desc/Cllms Page number 39>

The results show that the broyat, with various amounts, increases in a amount-dependent way: < RTI ID=0.0> - la< /RTI> concentration of the synthesized ATP, - total concentration of cellular nucleotides adenylic.

In addition, the energy load (EC) remained constant, representing a stable energy balance between nucleotides adenylic: < RTI ID=0.0>

ATP > ADP +Pi

ATP > AMP + Ppi< /RTI> (Pi: inorganic phosphate) These results are in perfect correlation with those obtained during the first 2 stages, and confirm well that the product involves a stimulation of the cellular energy metabolism.

Example 7 Study of the tolerance of a < RTI ID=0.0> broyat< /RTI> of vine

A study of cutaneous tolerance and eyepiece in vitro at summer realized on a purely preliminary basis for the development of cosmetic preparations.

These tests revealed a perfect local tolerance (cutaneous and ocular) of the broyat to the concentration of 0,3%.

The broyats of cells can be directly used to form cometic compositions with topic use.

One will give hereafter some examples, nonrestrictive, such compositions with topic use

< example; RTI ID=0.0> 8< /RTI> Dispersion of cells of vine élicitées and whole in a cosmetic base

The cells of vine are obtained as described in the < examples; RTI ID=0.0> 1A< /RTI> with < RTI ID=0.0> 11.< /RTI>

The cells of these examples were used separately or mixes some for the preparation of a cosmetic composition. The cells are dispersed after freeze-drying without to be crushed in the following base:

< Desc/Cllms Page number 40>

< RTI ID=0.0> - Eau< /RTI> deionized 85,61% - Mineral oil 9, 00% - cetyl Alcohol 3, 00% < RTI ID=0.0> - cetareth-20< /RTI> 0,75% - cells of vine 0,20% - fragrance 0, 15% < RTI ID=0.0> - carbomer< /RTI> 0, 10% < RTI ID=0.0> méthylchloroisothiazoline< /RTI> and méthylisothiazoline [kathon CG] 0,065% - sodium hydroxide (45%) 0,06 < RTI ID=0.0> %< /RTI> - hydroxyanisole butyl 0,06%
TOTAL 100,00%

The composition obtained shows a homogeneous dispersion of the cells in the cream and a very fine granulometry. The study of cleanliness showed the absence of germs and mushrooms as well as a remarkable stability of the composition.

The result obtained having been tested in a transcutanée study made it possible to see the passage of the active ingredients in particular the polyphenols through cutaneous fabric.

Example 9 Dispersion of cells of vine élicitées and crushed in a cosmetic base the cells of vine are obtained as described in the < examples; RTI ID=0.0> 1A< /RTI> with 11.

The cells of these examples were used separately or mixes some for the preparation of a cosmetic composition. The cells are dispersed after freeze-drying and crushing in the following base: < RTI ID=0.0> - eau< /RTI> deionized 85,61% - mineral oil 9,00% - cetyl alcohol 3,00% - cetareth-20 0,75% - cells of vine 0,20%

< Desc/Cllms Page number 41>

- fragrance 0, 15% < RTI ID=0.0> - carbomer< /RTI> 0, 10% < RTI ID=0.0> - méthylchloroisothiazoline< /RTI> and méthylisothiazoline < RTI ID=0.0> [kathon< /RTI> CG] 0,065% - sodium hydroxide (45 < RTI ID=0.0> %) < /RTI> 0,06 < RTI ID=0.0> %< /RTI> < RTI ID=0.0> - hydroxyanisole butylée< /RTI> 0,06 %
TOTAL 100,00%

The composition obtained shows a homogeneous dispersion of the broyat of cells in the cream and a very fine granulometry. The study of cleanliness showed the absence of germs and mushrooms as well as a remarkable stability of the composition. The result obtained having been tested in a transcutanée study made it possible to see the passage of the active ingredients in particular the polyphenols through cutaneous fabric.

Example 10 Dispersion of cells of vine élicitées and whole in a cosmetic base

The cells of vine are obtained as described in the < examples; RTI ID=0.0> 1A< /RTI> with < RTI ID=0.0> 11.< /RTI>

The cells of these examples were used separately or mixes some for the preparation of a cosmetic composition. The cells are dispersed after freeze-drying without to be crushed in the following base: < RTI ID=0.0> - eau< /RTI> 46, 89% - laureth sulphate of sodium < RTI ID=0.0> (25%) < /RTI> 36, 40% < RTI ID=0.0> - PEG-7< /RTI> glyceryl cocoate 2, 00% - laureth-2 1, 50% < RTI ID=0.0> - laureth-11 carboxylate< /RTI> of sodium 4, 00% - cocamidopropyl betain & acid benzoic 3, 48% - sodium chloride 1, 60% < RTI ID=0.0> - propylene glycol 1, 00% < /RTI> - perfume 0, < RTI ID=0.0> 13% < /RTI> < RTI ID=0.0> - PEG-40< /RTI> hydrogenated oil of beaver & propylene glycol & water 0, 50%

< Desc/Cllms Page number 42>

- oleth-10 0,50% - sodium 0,30% phosphate - phosphate disodic 0, < RTI ID=0.0> 08% < /RTI> - acid citric (50 < RTI ID=0.0> %) < /RTI> 0, 52% - sodium benzoate 0, 50% - cells of vine 0, 20% - acid salicylic 0, 20% - phénoxyéthanol 0, 20%

TOTAL 100,00%

The composition obtained shows a homogeneous dispersion of the cells in the cream and a very fine granulometry. The study of cleanliness showed the absence of germs and mushrooms as well as a remarkable stability of the composition.

The result obtained having been tested in a transcutanée study made it possible to see the passage of the active ingredients in particular the polyphenols through cutaneous fabric.

Example 11 Dispersion of cells of vine élicitées and crushed in a cosmetic base the cells of vine are obtained as described in the < examples; RTI ID=0.0> 1A< /RTI> with < RTI ID=0.0> 11.< /RTI>

The cells of these examples were used separately or mixes some for the preparation of a cosmetic composition. The cells are dispersed after freeze-drying and crushing in the following base: < RTI ID=0.0> - eau< /RTI> 46, 89% < RTI ID=0.0> - laureth< /RTI> sulphate sodium < RTI ID=0.0> (25%) < /RTI> 36, 40% < RTI ID=0.0> - PEG-7< /RTI> glyceryl cocoate 2, 00% - laureth-2 1, < RTI ID=0.0> 50% < /RTI> < RTI ID=0.0> - sodium laureth-11 carboxylate< /RTI> 4, < RTI ID=0.0> 00% < /RTI> - cocamidopropyl betain < RTI ID=0.0> & < /RTI> acid benzoic 3, < RTI ID=0.0> 48% < /RTI> - sodium chloride 1, < RTI ID=0.0> 60% < /RTI> - propylene glycol 1, 00% - perfume 0, 13% < RTI ID=0.0> - PEG-40< /RTI> hydrogenated oil of beaver

< Desc/Clms Page number 43>

& propylene glycol & water 0, 50% < RTI ID=0.0> - oleth-10 0, 50% < /RTI> - sodium 0,30% phosphate - disodic phosphate 0, < RTI ID=0.0> 08% < /RTI> - acid citric (50 < RTI ID=0.0> %) < /RTI> 0, 52% - sodium benzoate 0, 50% crushed cells of vine 0, 20% - acid salicylic 0, 20% < RTI ID=0.0> - phénoxyéthanol< /RTI> 0, 20 %
TOTAL 100,00%

The composition obtained shows a homogeneous dispersion of the broyat of cells in the cream and a very fine granulometry. The study of cleanliness showed the absence of germs and mushrooms as well as a remarkable stability of the composition. The result obtained having been tested in a transcutanée study made it possible to see the passage of the active ingredients in particular the polyphenols through cutaneous fabric.

Example 12 Creams - aqueous phase A < RTI ID=0.0>: eau< /RTI> demineralized associated a hydrating product - oily phase b: émulsifier + émollifiant + oil - phase C: conservative, perfume < RTI ID=0.0> - phase D: < /RTI> active product: broyat of dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder.

Example 13 Lotions containing only one aqueous phase a: demineralized water, propylene glycol, conservative, perfume and active product: broyat of dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder.

< Desc/Clms Page number 44>

Example 14 Shampoos containing only one aqueous phase A containing demineralized water, detergents, foaming, thickening, perfume and active product: broyat of dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder.

Example 15 Gel < RTI ID=0.0> one < /RTI> consider the hydrogels and the oléogels, obtained by addition to aqueous phase A or the oily phase B of agents of the type emulsifying and thickening.

- phase C: perfume, preserving < RTI ID=0.0> - phase D: broyat< /RTI> dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder.

Example 16 Solutions Solutions containing only one aqueous phase A primarily containing demineralized water, perfume, conservative and active product: broyat of dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder.

Example 17 Milks - aqueous phase a: primarily containing deionized water - oily phase b: oil + émulsifier + émollifiant - phase C: conservative + produced hydrating < RTI ID=0.0> - phase D: < /RTI> active product: broyat of dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder.

< Desc/Clms Page number 45>

In the examples given above, concerning the creams, gel or milks, the various phases A, B, C and D, in proportions which can vary between them according to the desired applications, is mixed in a usual way, as usually the Expert in this field carries it out.

Concerning the lotions, solutions and shampoos, the composition with topic use contains the various components, which one can vary the contents according to the applications, mixed in the only aqueous phase, as usually the Expert in this field carries it out.

The proportion of the broyat of dédifférenciées and élicitées cells of Vine, in the form of a viscous suspension or of a gel or an appreciably dry powder is a function of the nature of the composition with topic use and the desired application. It advantageously lies between 0,01 and < RTI ID=0.0> 5%, < /RTI> but can reach up to 25%.

Obviously, the invention is not limited to the examples of realization given above and it is possible to carry out the composition with topic use in other forms, such as oil, ointment, lacquers, make-ups (make-up foundation, powders, lipstick, pencil, will mascara, eye shadow) which also enter within the framework of the invention.

Also, the invention is not limited to the cells of Vine and can apply to other types of vegetable cells, since they can be obtained in form dédifférenciées and that they can undergo a elicitation leading to an accumulation of secondary metabolites in sufficient quantity to allow the biological activity of topic use.

In all the cases, one obtains a composition with topic use which contains a broyat dédifférenciées and élicitées vegetable cells, in the form of a viscous suspension or of a gel or an appreciably dry powder.

< Desc/Clms Page number 46>

Example 18 One repeated the < example; RTI ID=0.0> 1, < /RTI> by using dédifférenciées vegetable cells coming from vegetable species different or mixtures from different vegetable species. In these examples, one used barks, pips or seeds, roots, sheets, stems, buds, fruits, skin or cuticle to obtain dédifférenciées vegetable cells.

The following table shows the vegetable species used:
EMI46.1

< tb>	
< tb>	
< tb>	< example; SEP> 18 < SEP> Species < SEP> vegetable
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	With < SEP> Rosmarinus
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	B < SEP> Coffea
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	C < SEP> Cocoa
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	D < SEP> Mungo
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	E < SEP> Colchicum
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	F < SEP> Jasminum < SEP> + < SEP> Iris
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	G < SEP> Capsicum
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	H < SEP> Pilocarpus
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	
< tb>	Sequoia
< tb>	
< tb>	
< tb>	
< tb>	
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< tb>	

< tb> J < SEP> Solanum
< tb>
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> K < SEP> Chlorophytum
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< tb>
< tb>
< tb>
< tb>
< tb> L < SEP> Ginkgo
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< tb>
< tb>
< tb>
< tb>
< tb>
< tb> M < SEP> digitalis
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< tb>
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< tb>
< tb>
< tb>
< tb>
< tb> NR < SEP> Salvia
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< tb>
< tb>
< tb>
< tb>
< tb>
< tb> O < SEP> Taxus
< tb>
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> P < SEP> Papaver
< tb>
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> Q < SEP> Salvia < SEP> + < SEP> rosmarinus
< tb>
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> Pinks
< tb>
< tb>
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> tea
< tb>
< Desc/Cls Page number 47>

EMI47.1

< tb> T < SEP> Bétula
< tb>
< tb> Vine < SEP> + < SEP> citrus < SEP> + < SEP> ginko
< tb>

Example 19 antioxydante Activity of broyats of various vegetable species:

The anti-oxidizing activity of broyats of broyats resulting from various vegetable species was studied in vitro. We used a model of reconstituted skins < RTI ID=0.0> SKINETHIC (2), < /RTI> allowing to reveal this activity by proportioning of malondialdéhyde (MDA), after its induction by ultraviolet rays B. The skins were treated for each vegetable species by a single concentration of broyat with 1%.

< RTI ID=0.0> Lipoperoxidation< /RTI> caused the experimental conditions of example 3 were repeated.

The results are gathered in the table below:

EMI47.2

< tb> < tb> < SEP> Species < SEP> vegetable, < SEP> broyat < SEP> with < SEP> 1% < SEP> Variation < SEP> < SEP> MDA < SEP> (in < SEP> %)
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> by < SEP> < report/ratio; SEP> with the < SEP> witness
< tb>
< tb>
< tb>
< tb> < SEP> negative
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Witness < SEP> negative
< tb>
< tb>
< tb>
< tb> < SEP> Witness < SEP> positive < SEP> UVB < SEP> (150 < SEP> mJ/cm) < SEP> increase < SEP> of < SEP> 45%
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Cocoa < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> - 29 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Mungo < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> - < SEP> 22 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Colchicum < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> - SEP> -26 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Jasminum < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -17%
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Capsicum < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -43 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Pilocarpus < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm)
- 41 < SEP> %
< tb>

< tb>
 < tb>
 < tb>
 < tb> < SEP> Sequoia < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
 < SEP> -59%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Solanum < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
 < SEP> -19%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> Chlorophytum < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -
 53%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Ginkgo < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -
 52%
 < tb>
 < Desc/Cims Page number. 48>

EMI48.1

< tb>
 < tb>
 < tb> < SEP> Pinks < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150mJ/cm2) - 25%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Betula < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -30%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> digitalis < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
 < SEP> -33 < SEP> %
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Salvia < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -47%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Taxus < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm') -
 56 < SEP> %
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Papaver < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
 < SEP> -53 < SEP> %
 < tb>
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> Cannabis < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
 < SEP> - < SEP> 47%

< tb>
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Rosmarinus < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -27 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Coffea < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -34 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Argan < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -51 < SEP> %
< tb>
< tb>
< tb>
< tb>
< tb>
< tb> < SEP> Catharantus < SEP> broyat < SEP> with < SEP> 1%+ < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -59 < SEP> %
< tb>
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< tb> < SEP> Iris < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) -19%
< tb>
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< tb> < SEP> Datura < SEP> broyat < SEP> with < SEP> 1 < SEP> % < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -53 < SEP> %
< tb>
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< tb> < SEP> Gloriosa < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm) -12 < SEP> %
< tb>
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< tb> < SEP> Medicago < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -33 < SEP> %
< tb>
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< tb> < SEP> Asparagus < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> -47 < SEP> %
< tb>
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< tb> < SEP> Borago < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) -22 < SEP> %
< tb>
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< tb> < SEP> Reseda < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
< SEP> -13 < SEP> %
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< tb> < SEP> Amsonia < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2)
< SEP> -26 < SEP> %
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< tb> < SEP> Erythrina < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP>
mJ/cm2) < SEP> -21 < SEP> %
< tb>
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< tb> < SEP> Coleus < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) <
SEP> -53 < SEP> %
< tb>
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< tb> < SEP> Oenothera < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP>
mJ/cm2) < SEP> -17 < SEP> %
< tb>
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< tb> < SEP> Atropa < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm2) <
SEP> -23 < SEP> %
< tb>
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< tb> < SEP> Theobroma < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP>
mJ/cm) - 17 < SEP> %
< tb>
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< tb> < SEP> Glycine < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> mJ/cm) -
43%
< tb>
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< tb> psoralea < SEP> corylifolia < SEP> broyat < SEP> with < SEP> 1%+UVB150mJ/cm2 < SEP> - < SEP> 40 <
SEP> %
< tb>
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< tb> < SEP> vitex < SEP> negundo < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 <
SEP> mJ/cm2) < SEP> - < SEP> 42 < SEP> %
< tb>
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< tb> < will commiphora; SEP> wighii < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> 150 <
SEP> J/cm-51 < SEP> %
< tb>
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< tb>
 < tb>
 < tb>
 < tb> vanilla < SEP> planifolia < SEP> broyat < SEP> with < SEP> 1% < SEP> + < SEP> UVB < SEP> (150 < SEP> J/cm) - 14 < SEP> %
 < tb>
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 < tb> marrubium < SEP> vulgare < SEP> broyat < SEP> with < SEP> 1%+UVB150mJ/cm2 < SEP> - < SEP> 28 < SEP> %
 < tb>
 < Desc/Clms Page number 49>

EMI49.1

< tb>
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 < tb>
 < tb> pilocarpus < SEP> jaborandi < SEP> broyat < SEP> 1%+UVB150mJ/cm2 < SEP> - < SEP> 41 < SEP> %
 < tb>

The results obtained reveal a significant protection of the broyats with respect to the lipoperoxydation caused by rays UVB (150 < RTI ID=0.0> mJ/cm2) < /RTI> with the concentrations < RTI ID=0.0> of 1%.< /RTI>

Under the experimental conditions selected, the broyats appeared to present a significant anti-ridicalizing activity at the level of skins reconstituted SKINETHICO after 24 hours of contact. This activity was revealed by the proportioning of the MDA.

Indeed, proportionings of the MDA show that the broyats studied with the concentrations of 1% significantly protect the cells against the lipoperoxydation caused by the ultraviolet rays B (UVB 150 < RTI ID=0.0> mJ/cm2) < /RTI> by decreasing the rates of induced MDA.

In conclusion, the broyats of the various studied vegetable species present a anti-ridicalizing effect under the conditions of induction by the ultraviolet radiations B. It comes out from this test that the broyats present a significant antiradicalaire effect. Taking into account the model selected (reconstituted skin), it can right now be planned to use these broyats in preparations with topics use with the minimal amount activates of 0, < RTI ID=0.0> 1%.< /RTI>

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Example 20 Lipopéroxydation caused the experimental conditions of example 3 were repeated by using the combination of aromatic plants with other species.

The results are gathered in the table below:

EMI50.1

< tb>
 < tb> < SEP> Species < SEP> vegetable, < SEP> broyat < SEP> with < SEP> 1% < SEP> Variation < SEP> < SEP> MDA < SEP> (in < SEP> %) < SEP> by
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 < tb> < SEP> < report/ratio; SEP> with the < SEP> witness < SEP> negative
 < tb>
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 < tb>
 < tb> < SEP> Witness < SEP> negative
 < tb>
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 < tb> < SEP> Witness < SEP> positive < SEP> UVB < SEP> (150 < SEP> mJ/cm2) < SEP> increase < SEP> of < SEP> 45%
 < tb>
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 < tb>
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 < tb> < SEP> Jasminum < SEP> sanbac+ < SEP> Ginko < SEP> bilboa < SEP> broyat < SEP> - < SEP> 12 < SEP> %
 < tb>
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 < tb> < SEP> 1%+UVB150mJ/cm2
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 < tb> eucalyptus < SEP> punctata+ < SEP> psoralea < SEP> coryfolia < SEP> broyat-21%
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> 1%+UVB150mJ/cm2
 < tb>
 < tb>
 < tb>
 < tb> lavandula < SEP> angustifolia < SEP> + < SEP> Vitex < SEP> negundo < SEP> broyat < SEP> - < SEP> 17 < SEP> %
 < tb>
 < tb>
 < tb>
 < tb>
 < tb> < SEP> 1 < SEP> +UVB150mJ/cm
 < tb>
 < tb>
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 < tb>
 < tb> < SEP> citrus < SEP> silt < SEP> + < SEP> sequoia < SEP> broyat < SEP> - < SEP> 24 < SEP> %
 < tb>
 < tb>
 < tb>
 < tb> < SEP> I < SEP> % < SEP> +IJB < SEP> 1 < SEP> 50mJ/cm2
 < tb>

Example the 21 1A examples with 1I were repeated if they are only the dédifférenciées cells, élicitées and washed were dried in an atmosphere (for example of nitrogen) having a temperature of approximately < RTI ID=0.0> 30 C, < /RTI> so as to reduce the water content of the cells to respectively 5% in weight, 10% in weight and 15% in weight.

The membrane structure of the cell was thus preserved.

One then subjected the cells dédifférenciées élicitées, washed and dried to a crushing.

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Example 22 One repeated example 20, if it is not that one mixed the dédifférenciées élicitées, washed and dried cells (with the preserved membrane structure) with one or more excipients and/or composed active of a cosmetic composition before the stage of crushing.

The following list shows certain excipients mixed with the cells before their crushing.

- sodium laureth sulphate (aqueous solution for example with < RTI ID=0.0> 25%) < /RTI> - PEG-7 glyceryl cocoate - antioxydant (aqueous solution containing of the vitamin E) - laureth-2 < RTI ID=0.0> - sodium laureth-11 carboxylate < /RTI> < RTI ID=0.0> - cocamidopropyl < /RTI> betain < RTI ID=0.0> - betaine < /RTI> (glycinebetaine) - essential oil - propylene glycol - ethanol < RTI ID=0.0> - PEG-40 < /RTI> hydrogenated beaver oil - vegetable oil (oil of coconut, olive oil, etc) - essential oil - mixtures of one or several compounds quoted herebefore between them and/or with water.

*La quantity of excipients et/d' water added to the cells can vary for example between 10% of the weight of the cells dried to crush and 100% of the aforesaid weight, even more.

The use of one or active agents tensio with one or more oxidizing anti agents seems interesting to facilitate the crushing of the cells in particular

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membrane, to facilitate the release of phytoalexines attached or been dependent on a membrane, and to reduce or avoid any problem of degradation of compounds due to moisture. It is then possible to extract from this broyat of the phytoalexines by a stage of extraction (for example successive stages of extraction by means of ethanol and filtration).

This example is thus an example of process of obtaining phytoalexine (S), in which: < RTI ID=0.0> - on < /RTI> puts in a culture medium of the vegetable cells dédifférenciées, - after and/or during the culture, one elicit the cells dédifférenciées in the culture medium, < RTI ID=0.0> - on < /RTI> separate the cells dédifférenciées and élicitées of the culture medium, one subjects possibly the dédifférenciées cells élicitées to one or more washings, one dries advantageously, preferably one freeze-dries, the dédifférenciées and élicitées cells, < RTI ID=0.0> - on < /RTI> crush the dédifférenciées cells, élicitées so as to form a broyat, and < RTI ID=0.0> - on < /RTI> the broyat subjects to an extraction to extract one or more phytoalexines from the broyat, possibly after a stage of handing-over of the cells crushed in a medium, in particular an aqueous and/or alcoholic medium.

Example 23 One repeated the examples with < RTI ID=0.0> 1I, < /RTI> if they are only the dédifférenciées and élicitées cells were washed and dried (by means of a nitrogen current with < RTI ID=0.0> 30 C) < /RTI> to eliminate the water of washing present at external of the membranes of the cells and to reduce the water content presents in the cells, respectively of 0%, 10%, 25%, 50% and 75%. The cells were then crushed. The broyat thus obtained appreciably contains all the components present in the cells, with a water content either reduced, or corresponding to water present

in the normal cells (normal content water of the cells).